Milk Adultration: Methods of Detection &Remedial Measures

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Abstract— **Food is essential for sustenance of life. Adulteration of food cheats the consumer and can pose serious risk to health in some cases. Major food adulteration and contamination events seem to occur with some regularity, such as the widely publicized adulteration of milk products with melamine and the recent microbial contamination of vegetables across Europe for example. With globalization and rapid distribution systems, these can have international impacts with far-reaching and sometimes lethal consequences. These events, though potentially global in the modern era, are in fact far from contemporary, and deliberate adulteration of food products is probably as old as the food processing and production systems themselves.**

Index Terms— **Adulteration of food, globalization and rapid distribution systems**

I. INTRODUCTION

Consumer is the largest economic group and central point of all marketing activities. With the rise in the income of people, the quality, the quantity and the sophistication of the consumer goods has also increased. The market is literally overflowing with the new products based on intricate technology. It is very difficult for the consumer to select one food item because of misleading advertisements, improper media emphasis and food adulteration. As a result of these malpractices, the ultimate victim is a consumer, who innocently takes adulterated foods and suffers (1). A good buying behavior reflects philosophy about the nature of consumer and provides a logical means of organizing the vast quantity of information on variables that influence the buying practices. Buying practices involves the determination by market agencies of kind, qualities and quantities of goods desired by consumer. Buyer has to find out the desired qualities of goods sold at satisfactory prices. Buying consumes a great deal of time, energy and money. Effective buying requires a specialized knowledge of content of goods, their resources and their use(Kotler, 1990). Consumers have no choice except to face a wider variety of buying situations than in the past, one result is that there has been and will continue to be an increase in the number of consumer problems and disputes particularly involving consumer rights and legal protection. To meet these challenges successfully Consumers must arm themselves against these problems since they are not automatically protected by the working of the market. (2)

Therefore, a consumer's best defense is knowledge of his/her rights as a consumer and of the remedies which exist to resolve these problems when they occur. "Knowledge and awareness about adulterated foods, laws and its rights related to adulterated food is crucial in a society where technology heightens opportunities for perpetrators of fraud deception and misrepresentation".

Adulteration may be intentional or unintentional. The former is a willful act on the part of adulterator who intended to increase the margin of profit. On the other hand, adulteration may be incidental contamination, which is usually due to ignorance, negligence or lack of proper facilities. Adulteration is defined as "the process by which the quality or the nature of a given substance is reduced through.

Milk is an important source of nutrient required for growth in infants and children and for maintenance of health in adults. Milk is a perfect food, readily digested and absorbed. It is a sole natural food for infants and children. It is chiefly a valuable source of good quality protein, fat, carbohydrates, vitamins and minerals. Protein in diet supply the amino acids required for growth of infants and children. It is also required for maintenance of tissues in adults.

Milk is one of the products which can be adulterated in many ways affecting the quality of further dairy products. Extension of milk with a low value ingredient (watering of milk, milk of different species, addition of whey, etc.) also known as "economic adulteration" has been often practiced. (3)

Different methods were developed to detect fraudulent addition to milk of rennet whey, a by-product of the dairy industry. Assays developed for the analysis of casein/whey protein ratio included indirect methods, based on determination of certain protein fractions, and direct methods, which separate protein mixtures into components. Indirect determination of whey protein to casein protein ratio included polarographic and second and fourth derivative spectroscopy methods. Direct determination of protein fractions is based on effective but laborious electrophoretic, chromatographic and immunoturbidimetric methods. (4)

Recently, detection of fraudulent manipulation of milk with whey has been focused on the analysis of glycomacropeptide (GMP) also known as caseinomacropeptide (CMP). It is a bioactive 64 amino acids residues glycopeptide released enzymatically in whey from *k*-casein by the action of chymosin during cheese making GMP structure lacks aromaticamino acids and retains a net negative charge, even at pH 3 (5). Quantitative determination of GMP includes

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chromatographic methods, *e.g.* RP-HPLC and combined LC-MS, and electrophoretic methods, *e.g.* SDS-PAGE and capillary electrophoresis techniques (6).
For the analysis of powder milk adulterated with whey, European Commission adopted two methods of gel-filtration HPLC for GMP detection. (7)
New strategies based on immunochemical assays have been also developed to analyze GMP. ELISA assay targeting bovine GMP has been successfully employed. The company Operon has developed a rapid immunochromatographic test (Immunostick c-GMP) based on monoclonal antibodies against GMP. GMP identification has been used to detect fraudulent addition of whey to milk powders and UHT milks as well as to monitor the renneting process. Despite the fact that generally, whey addition in milk does not represent a health hazard it was shown that supplementation of infant formula with GMP enhance the absorption of trace minerals. Consequently, reduction of trace minerals in formulas is needed in order to avoid possible adverse effects of excess dietary intake because of supplemental GMP. (8)

II. MATERIALS AND METHODS-

A. Milk and milk drinks samples

Six commercial bovine milk samples from the national market were used (fresh, pasteurized, UHT, omega-3 UHT, condensed and milk powder) and four dairy beverage samples of cappuccino, chocolate, banana and strawberry flavors. Fresh milk sample was obtained from a farm in the neighborhood of Sibiu and the other milk/milk drinks samples were obtained from commercial sources (brands A, B, C, D, E, F and G). Nutritional values of the samples were obtained from the information given by the manufacturer. The milk powder sample was reconstituted with deionized water following the instruction given by the manufacturer.
Where necessary, total protein content was determined from nitrogen content by Kjeldahl method (crude protein: N x 6.38). (9)

B. Sample preparation

The procedure consists of a pre-treatment of samples with trichloroacetic acid (TCA) to precipitate casein and major whey proteins [10]. Precipitation was done with 20% TCA for 10 minutes at r.t. The precipitate was removed by centrifugation at 5000 *g* for 10 minutes at r.t. The supernatant was filtered on 5 µm low protein adsorption filter. Three dilutions were obtained (1/10, 1/100 and 1/1000 respectively) using the Tris buffer pH 7.2 with 0.1% sodium azide. All the reagents were of analytical grade purity.

C. GMP detection

Qualitative determination of GMP in selected samples was performed using the Immunostick c-GMP visual assay. Immunochromatographic sticks (purchased from OPERON S.A., Spain) which contain monoclonal antibodies specific for GMP and anti-GMP antibodies were dipped into solution samples diluted 1/1000. Development of a red band in addition to the control blue band on the reactive strips after 5 minutes was considered a positive result. (11)

III. OTHER METHODS FOR DETECTION OF MILK ADULTRATION-

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 have been delineated below:

A. Detection Of Removal Of Fat By Skimming

The following indicates this:
(i) Lower percentage of fat
(ii) Higher density reading
(iii) Higher ratio of SNF: fat.(12)

B. Detection of Added Waster

The following indicates this
(i) Lower percentage of fat.
(ii) Lower percentage of SNF
(iii) Lower density reading
(iv) Depression of freezing point.

Water is the most common adulterant and its presence can be detected by testing the freezing point of milk the AOAc Specifies a freezing point for normal milk of –0.550C and the percentage of d.d. water is calculated as follows:
Percentage of added water = 0.550- T x 100 =0.550
T is the freezing point depression (FPD) of suspected milk sample. FPD of pure milk is 0.550.
A tolerance of 3 percent is allowed, which is equivalent to specifying a minimum FPD for authentic milk of 0.53350C. The addition of preservatives and other soluble matters like sugar and salts decrease the freezing point of watered milk and thus escape the detection of adulteration.

C. 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 percent iodine solution is added and mixed. Appearance of blue colour indicates the presence of starch which disappears on boiling and reappears on cooling. (13)

D. 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. (14)

E. 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 or protein- free filtrate and add 1 ml. modified Barfoed reagent. Heat in boiling water bath 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.

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

G. 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 sodium hypochlorite (2%) and 0.5 ml phenol (5%) Heat in boiling water bath for 20 sec. A bluish colour forms immediately, which turns deep blue afterward.

Pure milk shows salmon pink colour which gradually changes to bluish after 2 hours. (15)

H. 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, concerted investigations need to be taken up in this direction as the menace of urea adulteration in milk is rising day by day. (10)

Test (i)

Take 5ml milk and add equal volume of 24 percent 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%), 0.5ml. sodium hydroxide (2%) and 0.5 ml phenol solution (5%) and mix.

A characteristic blue or bluish green colour develops in presence of added urea whereas pure milk remains colourless. (4)

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%) Appearance 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. (16)

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) (17).

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 (18).

J. 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 (19).

K. Detection Of Synthetic Milk

Take 5 ml milk in a test tube and add 0.2ml urease (20 mg. per ml) Shake well and then add 0.1 ml of BTB solution (0.5%) Appearance of dark blue colour indicates the presence of synthetic milk. The methods for detection of urea and synthetic milk are same; the only difference is appearance of dark blue colour in case of synthetic milk (20).

L. Detection Of Neutralizers In Milk

Neutralizers 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 percent 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. (14)

Test (ii) Determination Of pH:
The pH of milk to which neutralizers have been added is generally alkaline. The pH of such milk is always more than 8.0, which can be determined by using indicator dyes, pH paper or electrometrically using pH meter. (21)

Test (iii) Alkalinity Of Ash:
Neutralization 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 neutralize the alkalinity exceeds 1.20 ml, it indicates the presence of neutralizers in milk. (22, 23)

M. Detection OfColouring 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:
(a) Artificial colours
(b) Coal tar dyes
(c) Annatto
(d) Turmeric

Some of these dyes are permitted only in some dairy products but none in milk. Thee are often 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. (24)

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. (25)

Test (iii)
Add a few drops of hydrochloric acid to milk. Development of pink colour indicates azo (coaltar) dyes. (26)

N. 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 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.

O. Detection Of Formaldehyde

Formalin (40 percent 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 follow:
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. (27)

P. 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 percent solution of paraphenylenediamine. 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. (28)

Q. 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.
(i) 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.
(ii) Take 1 ml filtrate in a test tube and add 4 ml of diphenyl amine sulphate or diphenylbenzidine reagent Development of blue colour indicates the presence of nitrates. (29)

R. Detection Of Vegetable Fat

In synthetic 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:

1) Detection By Measuring Analytical Constants:

The adulteration of vegetable fat in milk can be detected by extracting the fat either by Rose- Gottlieb method or fat extracted in butyrometer (special butyrometer having both end open) and measuring its physico- chemical characteristics such as Butyrorefractometer (BR) reading, Reichert – Meissi and Polenske values. (30)

S. 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 5 ml water and shaking again. If colour in acid layer persists, sesame oil/ vanaspati is present. (31)

T. Detection Of Adulterants By Using Kits

Several test kits for detecting various adulterants viz urea, neutralizers, sucrose, glucose, pesticides antibiotics, aflatoxins have been developed in our country at National Dairy Research Institute, Karnal, Central food technological Research Institute, Mysore, PCDF, Lucknow and elsewhere . For detection of mastitis, simple strip test has been developed further M/s Gist Brocades, Netherlands, have developed Delvotest kits testing presence of antibiotics and sulpher drug residues in milk. (32)

IV. CAUSES OF ADULTERATION-

1. Demand And Supply Gap: More acute during summer due to low milk production and increased demand.
2. Physical Nature Of Milk: Aqueous and opaque nature of milk can accommodate many adulterants in milk.
3. Degraded Moral Society: Wrecked moral status coupled with passion for profiteering.
4. Spoiled Socio-Economic Structure: Poor persons engaged in the business do so to increase their income and raise socio- economic status. (9)
5. Perishable Nature Of Milk: The unscrupulous producers / traders use preservatives neutralizers etc. to prolong the shelf life of sub standard milk.

6. Low Purchasing Power Of Customer: Encourages the supplier to adulterate milk and sell at cheaper rate.
7. Unorganised Condition Of Dairy Industry: Most of the milk is procured and traded by unorganized dairies, which freely adulterate the milk.
8. Low Legal Standards And Their Improper Enforcement.
9. Lack Of Suitable, Rapid And Sure Tests: Consumers have no access to public analytical laboratories to get their samples analyzed. (33)

V. REMEDIAL MEASURES-

(1) There is need for rationalization of the standards prescribed under PFA Act. Buffalo milk, for which minimum requirement for fat % is 6 in most of the states, is hardly available as such in the market for sale. Either it is watered and sold as cow milk or admixed with cow milk and sold as mixed milk. In lieu of buffalo milk, full cream milk has been introduced containing minimum of 6.0% fat and 9.0% SNF.
(2) The PFA Act and Rules must be strictly enforced and offenders punished adequately. The manpower limitation, lack of adequate training to the food inspectors and apathy of consumers encourages the menace of adulteration.
(3) The adulterated substandard and injurious food stuffs (including dairy products) should be discouraged from trade. The enforcement of the act should be rigidly carried out particularly against unorganized dairies and small traders and vendors, which are the root causes of this malpractice.
(4) Certain discrepancies exist in the standards prescribed under PFA Act and ISI and Agmark standards. These anomalies should be rectified and a uniform standard should be prescribed.
(5) Rapid, reliable and inexpensive tests to detect various harmless and harmful adulterants should be worked out so that cases of adulterations are detected readily.
(6) The milk producers should be given incentives for clean milk production and should be encouraged to supply the milk to the registered village societies, milk unions and dairies. The various intermediaries should be eliminated.
(7) Special provision should be made for packaging and distribution / sale of dairy products. Most of the market samples of dairy products are stored under unhygienic conditions and sold loose without any specification with regard to the nature and content of the product. (34)
(8) Special provisions should be framed for rigorous control over the production, distribution and sale of milk and milk products including registration of premises where they are manufactured, maintenance of premises in a sanitary condition and maintenance of healthy states of human beings associated with the production, distribution and sale of such foods. (35)

VI. CONCLUSION-

Form the present study it could be concluded that low income group respondents were least educated, had low awareness about their rights and responsibilities and food adulteration. So this group needs to be armed with lot of information and training on the issues of food adulteration and ways to raise their voice when felt cheated. They had limited income, so they could not reach the standard items of their choice. On seeing such condition of consumer, our government has made sincere efforts to curb the fraudulent practices by enactment of various laws. It is highly unlikely that more legislation or increasing fines and jail terms alone will help reduce adulteration, particularly given the corruption that exists in the enforcement area and the low conviction rate. Greater consumer vigilance and action alone can help improve the situation. But such efforts are not fruitful unless consumers themselves are aware of their rights and responsibilities. Under these circumstances, consumer literacy is the need of the hour with special attention to low income groups who suffer the most.

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