Research Article

SCREENING OF ADULTERANTS IN MILK

IJCRR
Section: Healthcare
Sci. Journal
Impact Factor
4.016

Pradeep S.[1], P. Lakshminarayana[2], Varsha R.[3], Shriya K. Kota[3]

[1]Assistant Professor, Department of BT, B.M.S. College of Engineering, Bangalore, Karnataka, India; [2]Associate Professor, Department of MCA, B.M.S. College of Engineering, Bangalore, Karnataka, India; [3]UG Student, Department of BT, B.M.S. College of Engineering, Bangalore, Karnataka, India.

ABSTRACT

Milk is the most commonly adulterated consumable in India. The addition of urea, detergent, sugars and vanaspati oil to create synthetic milk render it unfit for consumption according to the standards that define the quality of milk. This paper investigates the different milk adulterants and two methods of detecting them. The chemical methods of testing and electrical conductance method were performed. It was found that urease test can detect as low as 0.2 g/L of urea present, iodine test for starch is able to detect 0.04g/L starch present and the sensitivity of Benedict's test is 5g/L. It was also observed that there was significant difference in conductance of adulterant containing milk from that of raw milk. Therefore these methods can be used for reliable detection of adulterants and can be incorporated into a device for easy detection of adulterants.

Key Words: Adulteration, Conductance, Chemical methods

INTRODUCTION

Adulteration is the act of addition of substances to a product that makes it unfit for consumption. These impurities are added to substitute the contents of a product at a cheaper rate to increase the quantity. Milk adulteration is one of the most common and old form of adulteration. This is because India is the largest country in milk production and consumption according to WSPA (World Society for the Protection of animals) and the National Dairy Development Board, India. As the population increases, the demand will increase because there will be more mouths to feed. To meet the exponentially increasing demand, adulteration is being employed on regular basis.

Adulteration not only includes the intentional addition or substitution of materials but also the incidental contamination during the process of preparation, storage and transportation. Adulterated food has adverse effects on heath because of the toxic nature of the substituting compounds or lack of compounds of nutritional value. [1]The most common adulterants added to milk are water, urea [2], starch, oils etc. Consumption of urea will lead to kidney failure, damages the heart and liver. A study in Varanasi showed that the majority of milk consumers are children and these children experienced headache, eyesight problems and diarrhoea due to large scale use of urea.[3]Excessive intake of starch may displace nutrients and contribute to obesity.[4]A 2007 report in the *Journal of American Heart Association* found that consumption of vanaspati elevates cholesterol levels in the body thus causing diabetes and coronary ailments. A national survey shows that almost 70% of our nation's milk is adulterated with detergent, neutralizers but impure water was the major contaminant. Water is the most common adulterant; dilution of milk with impure water not only reduces nutritional value to a great extent but also causes water borne diseases. To enhance SNF value of milk, detergents are added which on consumption may cause health hazards.[5]

Therefore, a need for methods to detect is entailed. Chemical method of detection is one of the various methods of detection. Here, the adulterant is detected by inducing a reaction with a particular compound thus producing a coloured compound whose appearance is enough to detect whether adulterant is present or not. Furthermore, OD can be read for calculating the concentration of adulterant in the sample. These chemical tests are very specific to a particular compound and hence false positive results will not be obtained. The sensitivity of the certain tests discussed below is very high. It has been found that urease test can detect as low as 0.2 g/L of urea present. [6]

Milk conducts electricity due to the presence of ionic minerals of which chloride and sodium ion are key players. The

Corresponding Author:
Pradeep S., Assistant Professor, Department of BT, B.M.S. College of Engineering, Bangalore, Karnataka, India;
E-mail: padees@gmail.com

Received: 23.03.2016 **Revised:** 19.04.2016 **Accepted:** 28.05.2016

conductivity can be used to detect added neutralizers and other adulterants in milk. [7]The conductance of milk has been used for many decades to measure the fat and protein content of milk. Current research is focused onto determining the quality of milk by measuring the electric admittance of milk.[8]The milk composition and rheology affects the conductivity. Presence of more fats decreases the conductivity of milk. Milk conductance measurement has also been used as a reliable method for detection of mastitis in cows. It is observed that there is a sharp increase in conductance of milk from cows infected with mastitis. Storage period and storage temperature also affects electric conductance. It was observed that storage at 10°C and for short periods maintains the good quality of milk. This is because the microbial flora present in the milk will expand and ferment milk releasing lactic acid that reduces pH of milk. The presence of lactic acid will increase conductance due to formation of lactate ion.[9]

MATERIALS AND METHODS

The most common impurities found in milk available in India are water, urea, detergent, starch, glucose and Vanaspati or vegetable oils.

1. Impurities in milk were detected by carrying out certain chemical tests according to the protocol given by Food Safety and Standards Authority of India. [10]

Table 1: Chemical tests for detection of adulterants

Adulterant	Test	Inference	Remarks
Urea	Urease test – take 2ml of milk sample and add 1ml of phenol red (indicator) and keep in water bath at 35°C for 5mins. Then add 0.5 ml of urease.	Appearance of Peach – trace amounts Reddish brown - low concentration Pink – moderate concentration Magenta – high concentrations	To increase SNF and lactometer reading.
Detergent	Shake 5-10ml of sample with equal volumes of water.	Formation of lather confirms presence of detergent	For colour improvement in milk and increase SNF
Starch	Iodine test – add a few drops of iodine solution.	Appearance of blue black colour indicates presence of starch	For thickening of milk and increase lactometer reading.
Glucose	Add 2 ml of milk sample and equal volume of benedict's reagent into a test tube. Keep in boiling water bath for 5 minutes and observe colour changes.	Appearance of Blue colour – no glucose present Green - trace amounts Yellow – low concentration Orange- moderate concentration Red – high concentration	Glucose is added to milk to increase consistency and taste.
Inverted sugar	Prepare yeast by mixing 3g dry yeast with 20 ml of distilled water. Let it stand for 20 minutes. Fill test tube 1/3 full with sample. Add 3 ml yeast suspension to it. Mix well .After 10 minutes carry out Benedict's test for reducing sugars.	Test results are similar to glucose test. Appearance of Blue colour - no glucose present Green - trace amounts Yellow - low concentration Orange- moderate concentration Red - high concentration	Is added along with glucose for enhancing taste.
Vanaspati	Take 3 ml of milk in a test tube. Add 10 drops of hydrochloric acid. Mix up one teaspoonful of sugar. After 5 minutes, examine the mixture.	The red colouration indicates the presence of Vanaspati in milk.	To increase fat in milk and SNF.
Water	Put a small amount of milk on smooth slant surface	Adulterated milk will not leave a trail and slides faster.	The milk is diluted to increase quantity and thereby decrease quality.
Synthetic milk	Rub a drop of milk between fingers. And also boil a small amount of milk and observe the colour change.	Gives soapy feeling on rubbing between the fingers and turns yellow on heating.	Synthetic milk is made by mixing urea, detergent, paints and oils etc

2. Preparation of standard graph:
   a) Starch standard graph: 0.008 g, 0.01g, 0.012g till 0.1g of starch were weighed and dissolved in 20ml of distilled water separately. 2ml of the starch solution was added to test tubes containing 2ml of milk sample each. To the test tubes few drops of iodine solution were added. The violet / blue black colour was read at 420nm.[11]
   b) Urea standard graph: 0.004g, 0.006g, 0.008g till 0.30g of urea were weighed and dissolved in 20ml of distilled water separately. 2ml of the urea solution was added to 2ml milk sample and 1ml of phenol red was added. The test tubes are incubated in water bath at 35°C for 5mins. Then 0.5 ml of urease was added. The absorbance was read at 670nm (according to enzyme assay protocol by Sigma Aldrich).
   c) Glucose standard graph: 0.1g, 0.12g, 0.14g till 1g of glucose were weighed and dissolved in 20ml of distilled water separately. 2ml of the glucose solution was added to 2ml milk sample and equal volume of Benedict's reagent was added into the test tubes. The test tubes were then incubated in boiling water bath for 5 minutes and absorbance was read at 550nm (according to protocol in Lab manual for biochemistry and immunotech).
3. DOE: The conductance was measured using multimeter. The electrodes were dipped in the beaker containing milk solution. The readings were taken at RT around 25°C. A comparative analysis was done.
   a) The conductance of raw milk was recorded initially followed by boiling the milk for 5 minutes and conductance of boiled milk was read.
   b) Small amounts (0.001g) of different adulterants were added and conductance was read.
   c) Dilution with water was done using raw milk and distilled water in different ratios like 1:1(1ml milk and 1ml water) and so on till 1:4. The conductance was measured.
   d) A milk sample was used to measure the conductance.

RESULTS

The above tests were performed on milk sample which tested negative for all tests. The tests mentioned above are highly sensitive and can detect even trace amounts of the respective adulterant present in milk.

Table 2: Results of chemical tests

Sl No	Name of Test	Observation	
1	UREASE TEST	The colour change was orange-yellow indicating the absence of urea.	
2	DETERGENT TEST	No lather formation indicates the absence of detergent in sample.	
3	IODINE TEST FOR STARCH	Yellow coloration was observed which indicates the negative result.	

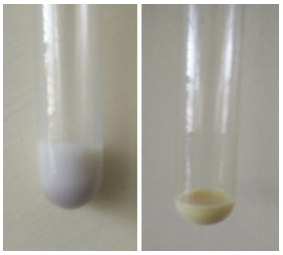
4	BENEDICT'S TEST FOR GLUCOSE AND INVERTED SUGAR	slight blue colouration of sample indicates negative result	
5	VANASPATI	No red coloration was observed indicating absence of vanaspati.	
6	SYNTHETIC MILK AND WATER	No soapy feeling on rubbing between the fingers and no yellow colouration on heating was observed. Sample didn't leave a trail and slid faster compared to raw milk.	

Table 3: Conductance Results

Sl No	Component	Conductance Measured (Volts)	Inference
1	Water 1:1 1:2 1:3 1:4	 0.098 0.086 0.018 -0.003	The conductivity decreases as the dilution (milk: water ratio) increases because the ion concentration decreases with increase in water amount.
2	Urea	0.030	Urea is a non conductor and in small amounts the conductance variation is insignificant.
3	Detergent	0.064	Addition of detergents increases the amount of ions in the solution due to its dissociation and thus increases conductivity.
4	Starch	0.031	Starch is a non conductor and hence in small quantities there exists minor difference in the conductance.
5	Dextrose	0.029	Similar to starch, dextrose also has trivial effect on conductance.
6	Vegetable oils	0.011	The oils decrease the conductance by increasing the viscosity of milk which doesn't allow for easy mobility of ions.
7	Milk sample	0.085	The sample is diluted and there might be presence of microbial flora.
8	Boiled raw milk	0.019	The conductance of boiled milk is lesser than raw milk due to destruction of microbial flora present in the raw milk.

DISCUSSION

The sensitivity of urease test was verified. Iodine test for starch is able to detect 0.04g/L starch present. The sensitivity of Benedict's test was found to be 5g/L. The standard graphs were generated which can be used for finding unknown concentration of the adulterant in samples.

The conductance of raw milk was found to be 0.032V. The sample was diluted with water but didn't contain any of the adulterants mentioned above. The conductance results show that the milk sample is 1:2 diluted. From the conductance and chemical test results it can be concluded that the milk sample is diluted and there was presence of microbial flora.

CONCLUSION

By performing the above simple tests conclusion can be drawn that the samples are adulterated or not. The chemical tests can be employed to effectively detect these common adulterants because of their high sensitivity. Conductance measurements can be used for qualitative analysis of adulterants. The above conductance tests reveal that it is a reliable method for detection of adulterants.

FUTURE SCOPE

Since adulteration of food is becoming a common practice due to exploding population in India, it is essential that consumers be aware of the methods for detecting these adulterants and most importantly about the ill effects on human health by short term and long term consumption. Keeping this in mind and by considering the tolerable level of the adulterants, a biosensor can be devised that incorporates the above tests for detection of adulterants. By a single input the adulterants can be detected based on pH change, colour change resulting due to induced chemical reactions and conductance measurements for qualitative analysis. Furthermore, research can be done to increase the sensor's sensitivity and repeatability by considering the extraneous factors like temperature etc. The sensor can be made such that it can be utilized by consumers at home for easy detection of these adulterants.

ACKNOWLEDGEMENT

We acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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