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Tanmay Hazra
Assistant professor, college of dairy science, Kamdhenu University Amreli Gujarat, India

Akshay Ramani
Akshay Ramani, M. Tech Scholar, College of dairy science, Kamdhenu University Amreli Gujarat, India

AS Hariyani
Assistant professor, college of dairy science, Kamdhenu University Amreli Gujarat, India

CHVK Sudheendra
Assistant professor, college of dairy science, Kamdhenu University Amreli Gujarat, India

Kunal Ahuja
Assistant professor, college of dairy science, Kamdhenu University Amreli Gujarat, India

VM Ramani
Principal& Dean, college of dairy science, Kamdhenu University Amreli Gujarat, India

Correspondence
Tanmay Hazra
Assistant professor, college of dairy science, Kamdhenu University Amreli Gujarat, India

A simple rapid chromogenic test for detection of palm oil adulteration in milk

Tanmay Hazra, Akshay Ramani, AS Hariyani, CHVK Sudheendra, Kunal Ahuja and VM Ramani

Abstract
Adulteration of milk is a big problem in India especially in lean season. Fraud milk traders, for earning more money, use to adulterate vegetable oil in milk, especially those oil has same fatty acid profile like milk fat. Palm oil has very similar fatty acid profile with milk fat, so incidents of adulteration of palm oil is increasing day by day. In past various techniques been applied for detection of oil adulteration in milk or milk fat, but most of these protocols or technique are time consuming and tedious, so it is not possible to apply those protocol under field conditions. Considering all facts a simple DPPH based chromogenic test been developed for detection of palm oil adulteration in milk or milk fat. The said protocol able to detect 5% level of palm oil adulteration in milk. Hence this said protocol is rapid very less expensive as well as no need of skill man power, thus this protocol could be used as a rapid method for detection of palm adulteration oil in milk.

Keywords: milk fat, palm oil, adulteration DPPH and antioxidant

Introduction
Mammals are able to secrete milk from mammary glands, to meet the needs of the neonate. The basic components of milk are, ie, protein, fat, lactose, minerals and water [1]; however those basic milk constituents use to be changed depending on the breeds, species, environment and milking periods etc [2]. The Cow is often considered as the "universal" animal for milk production and almost 85% of world's total milk production are contributed by Cows followed by Buffaloes [3]. However, India- the highest milk producing country in the world and most of the milk comes from buffalo followed by cow [4]. Milk is the basic food component for human civilization and in relation with Indian cultural aspect, milk is not only restricted as a stipulated diet but it has an integral relation with different religious activities. Milk and dairy industry is one of the growing industry in India and the demand of milk as well as milk products are getting popular day by day. However, due to high market demand and less supply of raw milk; the problem of milk adulteration getting a serious problem in Indian dairy industry [5]. In a survey conducted by FSSAI [6], reported that in Indian market, more than 68% milk are substandard and adulterated. The problem of adulteration is affected the whole parts of India. The common milk adulterants are water, urea, detergent, skim milk and oil [7].
In India, pricing policy of milk are mostly depends on fat content in milk; therefore recently to increase the fat content in milk, the fraud milk traders use to add vegetable oil in milk so that to increase the fat content in milk; hence the price of milk should be increased. Detection of vegetable oil in milk fat is very tough [8], especially those oil like palm oil that possess almost similar fatty acid profile to milk fat. Palm oil has a balanced fatty acid composition, with almost equal amounts of saturated and unsaturated fatty acids. The predominant fatty acids in palm oil are palmitic acid (C16:0, ranging between to 39-45%) and oleic acid (C18:1, ranging oils between 37-44%) [9].
However in past, various techniques [7, 10, 11, 12] have been applied to detect different vegetable oil including palm oil in milk or milk fat. But those techniques have some limitation like- time consuming, needs highly skill manpower or even expensive [13]. So all those techniques are very tough to use under field condition in routine quality control activities. So rapid test should very much essential for application in routine quality control activities. Considering all the facts stated above we have tried to develop a simple, rapid colorimetric test for detection of palm oil adulteration in milk or milk fat.

Materials and Methods

Procurements of milk

Mix milk samples were collected from local dairy farmers of Amreli. After collection of milk the fat content of milk were estimated by Gerber method [14].

Procurements of milk

Palm oil samples were procured from local market of Amreli.

Sample preparation

Oil were mixed with the milk @5%,10%,15% &20% respectively thereafter the milk and oil mixture were mixed with electric hand blender; so that no oil would be raised up on the top of the milk.

Preparation of DPPH solution

DPPH solution was prepared by mixing 50 mg of DPPH in 100 ml of Ethanol. Thereafter the solution was stored in 100 ml volumetric flask.

Gerber method for fat estimation

For estimation of fat in milk the Gerber method was applied according to FSSAI [14].The method is as follows-

Measure 10 ml of Sulphuric acid into a butyrometer tube, preferably by use of an automatic dispenser, without wetting the neck of the tube. Mix the milk sample gently but thoroughly and fill the milk pipette above the graduation line. Wipe the outside of the pipette and allow the milk level to fall so that the top of meniscus is level with the mark. Run the milk into the butyrometer tube along the side wall without wetting the neck, leave to drain for three seconds and touch the pipette's tip once against the base of the neck of the butyrometer tube. Add 1 ml of Amyl alcohol, close with a lock stopper, shake until homogeneous, inverting it for complete admixture of the acid. Keep in a water bath for 5 min. at 65±2°C taking care to have casein particles if any to dissolve fully, and centrifuge for 4 min. at 1100 rpm. The tubes should be put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge. Allow the centrifuge to come to rest. Remove the butyrometer tubes and place in water bath for 5 min. at 65±2°C. Read the percentage of fat after adjusting the height in the tube as necessary by movements of the lock stopper with the key. Note the scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid. When readings are being taken hold the butyrometer with the graduated portion vertical, keep the point being read in level with the eye, and then read the butyrometer to the nearest half of the smallest scale division.

Chromogenic test

After estimation of milk fat by Gerber method, 1 ml of fat from the butyrometer was kept in a test tube. There after 2 ml of DPPH solution were added in that test tube and kept it for 30 seconds; thereafter observe the colour. For checking the repeatability the test was performed 50 times.

Result and Discussion

DPPH (2, 2-diphenyl -1- picrylhydrazyl), is a dark-colored crystalline powder of stable free radical molecules. DPPH assay mainly use for study the antioxidant study [15]. Whenever, DPPH is mixed with a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour and pale yellow colour use to produce due to presence picryl group [16].

Initially we have tried different concentration (20, 30, 40 & 50 mg /100ml) of DPPH solution and we observed that 50mg of DPPH solution gave the best results, so this concentration was selected for further study.

It was observed in Plate (1) that the reaction of fat samples with DPPH (50 mg/100ml) solution; fat from pure milk turned to violet (pale yellow colour tinge still present), however the fat from the milk samples adulterated with 5,10,15 &20% palm oil turned to pale yellow colour. For checking the repeatability of this protocol; fifty times this test were conducted and same results were observed.

Milk fat contents some antioxidant components like phospholipid and beta carotene [2]. Hence it was observed in plate (1) that for pure milk fat, the intense violet colour was not observed but a pale tinge yellow colour was observed. That clearly represent the antioxidant activity of milk fat due to phospholipid and beta carotene. However, it was also observed in plate (1) that the milk containing palm oil; the violet colour was turned to yellow colour. Palm oil is a very good source phytonutrients like vitamin E, carotene, Phytosterol, phenolic compound and phospholipid [17] and those phytonutrients are very good source of antioxidants [18]. Therefore these antioxidants are able to scavenge free radicals actively, hence colour of adulterated fat samples turned violet to pale yellow colour with reaction to DPPH. This said protocol is rapid sensitive and even able to detect 5% level of palm oil adulteration in milk.

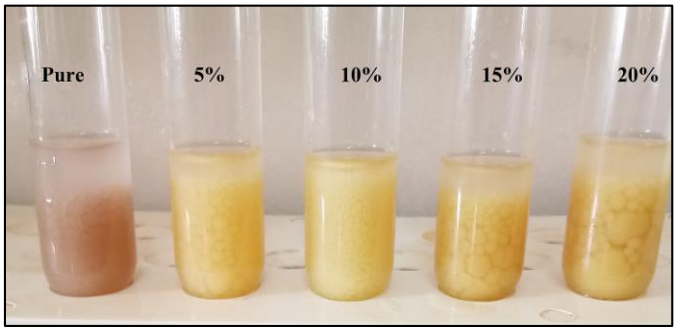

Plate 1: DPPH based chromogenic test for detection of palm oil in milk

(Pure-Pure milk fat, 5%- milk +5% palm oil,10%-milk+10% palm oil,15%- milk+ 15% palm oil,20% -milk+ 20% palm oil.)

Conclusion

Detection of foreign oil or fat, in milk fat is that like detection of tap water in river water. Here a simple rapid DPPH based chromogenic method was develop for detection of palm oil in in milk. Using this said simple protocol even 5% of palm oil adulteration in milk could be detected. Same results were observed after 50 trials. Hence this simple test could be recommended for regular quality control lab for detection of palm oil in milk.

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