Qualitative test for the detection of extraneous thiocyanate in milk

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Abstract Thiocyanate is one of the three components of lactoperoxidase system for preserving the milk under the conditions where chilling facilities do not exist due to economic and technical reasons. In 1988, International Dairy Federation (IDF) has recommended the levels and the code of practices for the application of LP-system. As the excessive use of thiocyanates beyond the recommended limits can lead to human health hazards, its rapid detection in milk at the reception dock of dairy, where milk is tested for quality before its acceptance or rejection, is necessary. For simple and rapid detection of added thiocyanates in milk, two types of qualitative tests have been developed in this study, of which one is applicable directly to milk and the other is applicable to TCA filtrate of milk. The results of the test reveal that as low as 0.6 ppm of added thiocyanate (equivalent to 1 ppm of potassium thiocyanate) can be detected. The orange to orange-red color obtained

in the test solution can be considered the basis of rejecting milk assuming that it has added levels of thiocyanate.

Keywords Thiocyanate · Lactoperoxidase system · Rapid detection · Qualitative test · Milk

Introduction

Milk and milk products have made significant contributions to human nutrition since the earliest civilization. Milk is a complete food, readily digested and absorbed. It is a sole natural food for infants for first few months of life as it is chiefly valuable source of good quality nutrients such as fat, proteins, lactose, minerals and vitamins which are present in highly desirable proportions. However, during transportation, from the point of its production to the place of processing, it undergoes microbial metabolism leading to breakdown of milk components culminating into rise in acidity. As the acidity of milk rises too high, it becomes unacceptable and unfit for processing. Cooling is the most common method (Claesson 1992; Lambert 2001) used to stop or retard deterioration of milk, but it is not possible in under-developed and developing countries because of problems related to electricity, lack of capital, high operational cost and poorly developed road system. Hence, the producers or the middlemen attempt to add various neutralizers such as sodium hydroxide, sodium bicarbonate, sodium carbonate, calcium hydroxide to neutralize the acidity produced. Apart from these, preservatives such as, formaldehyde, hydrogen peroxide, etc. are also added to prolong the life of milk before it is accepted at the milk processing plant. However, such practices of adding preservatives, neutralizers etc. is not permitted as per food laws (FSSAI Rules 2011 formerly known as PFA Rules 1955). Moreover, these additives affect the quality of milk during processing and may give rise to certain health hazards.

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Although, India has become the leading milk producer in the world, the quality of milk and milk products still remain a challenge for the dairy industry in order to be fit in the global competitive market.

An alternative method comprising of Lacto-peroxidase (LP) system (IDF 1988; FAO 1999) for increasing storage stability of milk at high ambient temperature is recommended in tropical regions where refrigeration of milk is not feasible. This method makes use of naturally occurring antibacterial system (LP system) in milk which is activated by increasing the concentrations of two components i.e. thiocyanate and hydrogen peroxide. Some work on the activation of LP system has also been done in India and abroad (Fonteh et al. 2005; Kumar and Mathur 1989a, b, c; Zhou and Lim 2009). However, it is not yet permitted by the food regulatory authorities. But some unscrupulous people in the trade, in order to prevent the rejection of their poor quality milk, have started taking advantage of the situation and incidences have come to the notice that thiocyanates are added irrationally to milk with or without hydrogen peroxide. Such a practice of addition of thiocyanates to milk is not only against food laws but it can also cause hazardous effect on the health of consumers, as thiocyanates are potent anti-thyroid substances which can lead to hypothyroidism when ingested at higher levels.

Levels of thiocyanates higher than the normal values reported for milk gives an indication of misuse of the components of LP-system. In 1988, International Dairy Federation (IDF) has given code of practice for the preservation of raw milk by the lactoperoxidase system. In their guidelines for its intended utilization, IDF has clearly stated that LP-system should be used in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities for maintaining the quality of raw milk. To have a check on the controlled usage of the LP-system, IDF (1988) have described a method for the quantitative analysis of thiocyanates in milk. However, such analysis can be done only in the quality control laboratory. There is need for the rapid detection of thiocyanates in milk at the reception dock of the dairy where milk is received and tested for its acceptance or rejection on the basis of its quality. This research paper deals with the qualitative test for the rapid detection of added thiocyanates in milk before further processing of milk in the plant.

Materials and methods

Collection of milk samples

Fresh pooled raw milk samples used for the present study were collected separately from cows and buffaloes from the cattle yard of the National Dairy Research Institute, Karnal, Haryana, India where animals are kept under identical conditions of feeding and management.

Preparation of reagents

Ferric nitrate reagent It was prepared by dissolving 16.0 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (LOBACHem Pvt. Ltd., Mumbai, India) in 50 mL of $2 \text{ mol.L}^{-1} \text{HNO}_3$ followed by making the volume to 100 mL with distilled water. The solution was filtered through Whatman filter paper No.1 or 4 and stored in dark or in amber colored volumetric flask.

Trichloroacetic acid reagent (20 %): It was prepared by dissolving 20 g of TCA (Fischer scientific, Mumbai, India) in distilled water and making the final volume to 100 mL with distilled water followed by filtration.

Development of a qualitative test for rapid detection of thiocyanates in milk

For the rapid qualitative detection of thiocyanates in milk, two types of tests were developed, of which one was applicable to TCA filtrate and the other was applicable directly to milk, as described below.

Fig. 1 Development of qualitative test for rapid detection of thiocyanates in milk: Test applicable to TCA filtrate

Fig. 2 Development of qualitative test for rapid detection of thiocyanates in milk: Test applicable directly to milk **a** with filtration **b** without filtration

Preparation of samples for both the tests

To the freshly drawn raw milk samples (control) of cows and buffaloes, thiocyanate contents @ 0, 0.6, 1.2, 1.8, 2.4, 3, 3.6,

4.2 and 4.8 mg/L were added. In terms of ppm, these levels amounted to 0, 0.6, 1.2, 1.8, 2.4, 3, 3.6, 4.2 and 4.8 ppm (equivalent to concentrations of potassium thiocyanate as 0, 1, 2, 3, 4, 5, 6, 7 and 8 ppm milk, respectively).

Fig. 3 Qualitative test for detection of thiocyanates in cow milk (applicable to TCA filtrate)

Fig. 4 Qualitative test for detection of thiocyanates in buffalo milk (applicable to TCA filtrate)

Fig. 5 **a** Qualitative test for detection of thiocyanates in cow milk (applicable directly to milk). **b** Qualitative test for detection of thiocyanates in cow milk (applicable directly milk with subsequent filtration)

Fig. 6 **a** Qualitative test for detection of thiocyanates in buffalo milk (applicable directly to milk). **b** Qualitative test for detection of thiocyanates in buffalo milk (applicable directly to milk with subsequent filtration)

Test applicable to TCA filtrate

Control as well as thiocyanate added milk samples (6 mL) were precipitated with 3 mL TCA (20 %) and filtered through Whatman no. 40 filter paper. To a clear 1.5 mL filtrate, 1.5 mL of Ferric nitrate reagent was added (Fig. 1). For blank, distilled water was taken instead of milk. Intensity of color in case of blank, control and thiocyanate added milk samples was used for differentiation and the criteria for qualitative detection was described by change in color from blank (no color) to the control (yellow color) and to the samples with added levels of thiocyanates (orange to orange-red color).

Test applicable directly to milk

To control as well as thiocyanate added milk samples (5 mL), equal quantities of Ferric nitrate reagent (5 mL) was added

(Fig. 2a, b). For blank, distilled water was taken instead of milk. Here also, the intensity of color in case of blank, control and thiocyanate added milk samples were used for differentiation. For more clarity, the milk samples after developing color were filtered through Whatman no. 40 filter paper and criteria for qualitative detection was described by change in color, both in milk as well as its filtrate, from blank (no color) to control (yellow color) and to the samples with added levels of thiocyanates (orange to orange-red color).

Results and discussion

Qualitative detection of thiocyanates in milk

In order to have a simple and rapid qualitative detection of thiocyanates in milk, two types of tests were conducted, of

Table 1 The order of colour intensity for detection of thiocyanate in milk (test applicable to TCA filtrate and directly to milk)

Sr. No.	Sample	Level of thiocyanate added (ppm)	Hue and intensity of colour
1.	Blank	–	Transparent
2.	Control (milk)	–	Yellow (with slight orange tinge)
3.	Test sample ^a	1	Yellowish-orange
4.	Test sample ^a	2	Orangish-red
5.	Test sample ^a	3	Orangish-red
6.	Test sample ^a	4	Orangish-red
7.	Test sample ^a	5	Red
8.	Test sample ^a	6	Red
9.	Test sample ^a	7	Red
10.	Test sample ^a	8	Red

^a Test sample- contains milk and added thiocyanate

which one was applicable to TCA filtrate and the other was applicable directly to milk.

Test applicable to TCA filtrate

Intensity of color in case of blank (colorless), control (yellow) and thiocyanate added milk samples (orange to orange-red) was differentiated and the criteria for qualitative detection was described by change in color from blank to control and to the samples with added levels of thiocyanates. The results obtained are presented in the Figs. 3 and 4 for cow and buffalo milks, respectively.

It was observed (Figs. 3 and 4) that the solution was colorless in case of blanks, and it was yellow with slightly orange tinge in case of solutions obtained from pure milk samples (cow and buffalo). Whereas, the solution obtained from cow and buffalo milks with added levels of thiocyanates showed clearly orange-red color with almost equal intensity for milks of both the species (cow and buffalo). With increasing concentration of added thiocyanates, the color intensity of the solution also increased. The solution was almost intense red at higher concentrations studied. Figures 3 and 4 showed that the color imparted by as low as 0.6 ppm of added thiocyanate (equivalent to 1 ppm of potassium thiocyanate) could be clearly differentiated from the color imparted by thiocyanates present naturally in milk.

Test applicable directly to milk

In this case also, the intensity of color in case of blank (colorless), control (yellow) and thiocyanate added milk samples (orange to orange-red) was used for differentiation. For more clarity, the milk samples after developing color were filtered through Whatman no. 40 filter paper. After filtration also, the criteria for qualitative detection was described by change in color from

blank to control and to the added levels of thiocyanates. The results obtained before and after filtration stage are presented in the Figs. 5a, b and 6a, b for cow and buffalo milks, respectively.

The Figs. 5a and 6a revealed that the solution was colorless in case of blanks, and it was yellow with slightly orange tinge in case of solutions obtained from pure milk samples (cow and buffalo), although the solutions were not very clear when the reagents were added directly to milk and subsequently not filtered. With increasing concentration of added thiocyanates, the color intensity of the solution was also increased. The solution was almost intense red at higher concentrations studied. Figures 5a and 6a showed that the color imparted by as low as 0.6 ppm of added thiocyanate (equivalent to 1 ppm of potassium thiocyanate) could be clearly differentiated from the color imparted by thiocyanates present naturally in milk.

Figures 5b and 6b showed that the solution was colorless in case of blanks and it was yellow with slightly orange tinge in case of solutions obtained from pure milk samples. However, the solutions were very clear in this case when the reagents were added directly to milk and subsequently filtered. The solution obtained from cow and buffalo milks with added levels of thiocyanates showed orange–red color with almost equal intensity for the two species. Higher the concentration of added thiocyanates, more was the intensity of color of the solution. The solution was almost intense red at higher concentrations studied. Figures 5b and 6b showed that the color imparted by as low as 0.6 ppm of added thiocyanate (equivalent to 1 ppm of potassium thiocyanate) could be clearly differentiated from the color imparted by thiocyanates present naturally in milk.

The present study revealed that the thiocyanates added to milk can be easily detected by the 2 types of qualitative tests developed. On comparing the two tests, it was inferred that the second test which was directly applicable to milk involving addition of ferric nitrate reagent and subsequent filtration, was better for its application as platform test to be used at the reception dock where milk is tested for acceptance or rejection based on its quality. As low as 0.6 ppm of added thiocyanate (equivalent to 1 ppm potassium thiocyanate) could be easily detected by the qualitative tests developed. However, in the quantitative detection of thiocyanates in milk by the IDF (1988) method, the detection limit has been reported to be 1 to 2 ppm of thiocyanates.

It was clearly observed in the study that the control sample (natural cow and buffalo milk) showed only the yellow color with slightly orange tinge, whereas the samples with added levels of thiocyanates showed orange to orange-red color. This shift of color intensity was visible even at the lowest level of thiocyanate added (0.6 ppm of thiocyanate equivalent to 1 ppm of potassium thiocyanate) to both types of milk samples (cow and buffalo). It was construed that the yellow color with slightly orange tinge observed in case of natural cow and buffalo milk samples may be not only because of the natural thiocyanates but because of some other unidentified natural

components of milk which might be contributing to the yellow color with the reacting reagents used in the experiment, apart from the slightly orange tinge imparted by the traces of natural thiocyanates upon reaction with reacting reagents.

It implies that with the addition of extraneous thiocyanate there is suppression in the intensity of yellow color with the concomitant increase of orange to orange-red color. Therefore, the orange to orange-red color given by as low as 0.6 ppm of thiocyanate (equivalent to 1 ppm of potassium thiocyanate) can be taken as the criteria for declaring the milk as thiocyanate added extraneously (thiocyanate positive) and ultimately rejecting the milk (Table 1).

Summary and conclusions

Two types of qualitative tests were developed in the study for simple and rapid detection of added thiocyanates in milk, of which one was applicable to TCA filtrate and the other was applicable directly to milk. On comparing the two tests developed for qualitative detection of thiocyanates, the second test which was directly applicable to milk and involved the addition of ferric nitrate reagent and subsequent filtration, was considered to be better for its application as platform test to be adopted in dairy industry under the field conditions where milk is tested for acceptance or rejection based on its quality. The study revealed that as low as 0.6 ppm of added thiocyanate (equivalent to 1 ppm potassium thiocyanate) can be easily detected.

Based on the color shown by the developed tests, it can be recommended that if the test shows orange to orange-red color

then such milk should not be accepted assuming that the milk has added thiocyanates.

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