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Research Article

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Development of a Rapid Qualitative Test Using Ferroin Dye for Detection of Rancid Ghee Mixed with Fresh Ghee

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Abstract

In the present study, a rapid ferroin dye-based test was developed for detection of rancid ghee mixed with fresh ghee. For assessing the ability of ferroin dye to detect the presence of rancid ghee mixed with fresh ghee, 40% (v/v) treated samples were tested. The developed method was optimized using various solvents and amounts of ghee samples as well as various temperatures and durations of heating during the experiment. For determining the limit of detection (LOD) of the developed test, 10, 20, 30 and 40% (v/v) rancid ghee samples were mixed with fresh ghee. The ferroin dye test was able to detect the presence of rancid ghee when mixed with fresh ghee at a level of 10%. Based on different levels of adulteration of rancid ghee in fresh ghee, a color chart was prepared for use in a ready platform test for the dairy industry.

Keywords

Rancid ghee, Ferroin dye, Adulteration, Quality control

Introduction

Ghee clarified milk fat, which is almost anhydrous. It is majorly consumed in India and subcontinent countries. It is manufactured by clarification of milk fat at 110 to 120 °C [1]. It is by far the most ubiquitous indigenous dairy product in India and prominent in the hierarchy of Indian foods. Ghee is a rich source of energy (9 kcal/g), fat-soluble vitamins (A, D, E and K) and essential fatty acids. It has a pleasant, nutty, slightly cooked or caramelized flavor and rich aroma [2]. The flavor is best described as a lack of oiliness or blandness, sweet rather than acidic. Ghee has a long shelf-life at ambient temperature. In India, it is considered an excellent medium for cooking; about 80% of ghee produced is used for this purpose. In addition, ghee is used on numerous auspicious occasions such as religious ceremonies. Therefore, in India, ghee enjoys a supreme status [2].

Chemically, ghee is a complex lipid comprising a mixture of glycerides, free fatty acids, phospholipids, sterols, sterol esters, fat-soluble vitamins, carbonyls compounds, hydrocarbons and carotenoids. Glycerides constitute about 98% and of the remaining constituents, sterols account for about 0.5% [2]. Ghee deteriorates through rancidification which may occur through hydrolytic and/or oxidative routes [3]. Oxidative rancidity is the major pathway by which ghee undergoes deterioration. The spoilage of ghee is of major concern as it is one of the costly dairy products. Deterioration of ghee affects not only the economic value of the product but also its appetizing flavor, reducing its palatability [4, 5].

The disposal of rancid and poor-quality ghee is problem for manufacturers who can be tempted to dispose of rancid ghee by partly replacing good-quality ghee with rancid ghee. Since the market price of good-quality ghee is higher than that of poor-quality ghee, mixing it with poor quality ghee may reduce economic losses [1]. However, consumption of such ghee containing rancid ghee may lead to health complications as oxidation of ghee produces various toxic compounds and consumption of such products can lead to diarrhoea, poor growth rate and promotion of tumor growth [6-10]. There are several physical, chemical and instrumental methods available for detection of foreign fats/oils as well as other cheaper materials (e.g. starch, sesamolin, dyes and synthetic colors) in ghee. In general, qualitative tests based on chemical reactions are considered to be simple, cheap, reliable, and easy to perform as rapid platform tests. When redox dyes react with oxidized components in a fat sample [11, 12], the developed color can be used as a measure of the extent of oxidation. There is potential to develop a rapid dye-based qualitative test for assessing the presence of rancid ghee in fresh ghee; however, no attempts have been made in this direction. Thus, the present study was taken up with the aim of developing rapid qualitative test for detecting presence of rancid ghee in fresh ghee. Furthermore, no attempts have been reported to develop such a test for other edible oils and fats.

Materials and Methods

Preparation of ghee

White butter was obtained from a commercial dairy plant, Amul Dairy, Anand, Gujarat. Ghee was prepared by creamery butter method in which butter was clarified by continuous stirring at 120 °C for 5 min. The ghee was filtered through four folds of muslin cloth. Three replications were performed. Fresh ghee was used as control.

Rancidity induction in ghee samples

To induce rancidity, samples of fresh ghee were stored at 80 ± 2 °C in a hot air oven. Changes in peroxide value of the samples were measured at 48 hour intervals. The samples were also monitored for changes in flavor by sensory evaluation using a 9-point hedonic scale. Storage at 80 ± 2 °C was continued until the flavor score was < 6 and/or the peroxide value was > 3.6 meq O_2/kg fat; the sample was then considered rancid.

Preparation of mixtures of rancid and fresh ghee

Rancid ghee was added to fresh ghee at 10, 20, 30 and 40% (v/v). The samples containing rancid and fresh ghee are referred to as treated samples. For evaluating the ability of ferroin dye to detect the presence of rancid ghee, 40% (v/v) treated samples were tested. These samples were also used for optimization of the method. For determining the limit of detection of rancid ghee in fresh ghee, 10, 20, 30 and 40% (v/v) treated samples were used. The samples spiked with rancid ghee with the lower percentages (2 to 10%, v/v) in preliminary trials and no clear color differentiation between control and treated sample was observed. But this test can be detected at 10% and above level of adulteration.

Storage of ghee samples

The control and treated ghee samples were stored at 80 ± 2 °C in a hot air oven and the development of the color in the ferroin dye test was observed at 48 hour intervals. Comparisons were made between the control and treated samples. The changes in the peroxide value and flavor score were determined. Storage was continued until the flavor score was < 6 and/or peroxide value of the control sample was> 3.6 meq O_2/kg fat.

Ferroin dye test

The ghee sample (5 mL) was placed in a clean and dry test tube and 5 mL of methanol was added and mixed well to dissolve the sample. One drop of 0.025 M ferroin dye (Loba Chemie Pvt. Ltd., Mumbai, India) was added and mixed in using a vortex mixer. The test tube was heated at 55 °C for 10 min and the development of the color in upper layer was observed.

Optimization of the ferroin test

For optimization of the ferroin test, the following parameters were studied.

1. Type of solvent (methanol, chloroform and chloroform: methanol mixture (7:3)) and amount of selected solvents (2, 5, 8 and 10 mL).
2. The amount (2, 5, 8 and 10 mL) of ghee used.
3. Temperature (37, 45 and 55 °C) and duration (5, 10 and 15 min) of heating.
4. Peroxide value.

The peroxide value of ghee was determined by the iodometric method as described by the Indian Standards Institution [13]. The ghee sample (1 g) was placed in a 150 x 25 mm test tube and 1 g of potassium iodide and 20 mL of the solvent mixture (glacial acetic acid-chloroform :: 2:1) were added. The contents were heated to boiling within 30 s in a boiling water bath and allowed to boil for not more than 30 s. The test tubes were transferred to a 250 mL conical flask containing 20 mL of freshly prepared 5% potassium iodide solution. The test tube was rinsed well with about 25 mL of distilled water and all washings were transferred to the above flask. The contents were titrated against 0.002 N sodium thiosulphate solutions using 2 mL of starch (1%) indicator. A blank was performed without taking ghee sample.

The peroxide value of ghee was calculated as meq peroxide oxygen/kg of ghee

Peroxide value (meq of peroxide oxygen/kg of ghee) = 2(S-B)/W

where S = volume in mL of 0.002 N sodium thiosulphate for sample; B = volume in mL of 0.002 N sodium thiosulphate without sample (i.e. blank) and W = weight in g of sample.

Sensory evaluation

All the samples of ghee made in laboratory were evaluated for their sensory characteristics on a 9-point hedonic scale by the panelist of 10 persons. The panelist, who were academic staff (aged 30 to 56) at SMC College of Dairy Science, Anand

Agricultural University, Anand, India. The panelist were experience persons and since long they have been working on oxidative rancidity of the ghee. The sensory evaluation was carried out in which odour (rancidity) was considered by the trained panelist. The treated samples were provided along with the fresh samples for better comparison. Each panelist evaluated the ghee for flavor (9, like extremely; 5, neither like nor dislike; and 1, dislike extremely).

Statistical analysis

The collected data were subjected to statistical analysis based on a two-factorial completely randomized design and critical difference test at 5% ($p < 0.05$) for comparison of the means for different treatments [14]. SPSS Statistics 17.0 was used for the analysis of data. Three replications were carried out.

Results and Discussion

The changes in peroxide value of ghee samples during storage at 80 ± 2 °C are shown in table 1. The peroxide values increased significantly during storage. The changes in flavor score of ghee samples during storage at 80 ± 2 °C are shown in table 2. The flavor score decreased significantly during storage.

Table 1 : Changes in peroxide value of ghee samples obtained upon storage at 80 ± 2°C.

Treatments	Storage time (Days)						Mean
	0	2	4	6	8	10	
Control	0.2 ± 0.01	1.3 ± 0.02	1.3 ± 0.03	3.8 ± 0.22	3.4 ± 0.26	4.7 ± 0.56	2.4
Treated (40%)	1.5 ± 0.09	2.7 ± 0.06	3.5 ± 0.17	5.0 ± 0.07	9.5 ± 0.70	11.0 ± 0.86	5.5
Means	0.9[a]	2.0[a]	2.4[a]	4.4[b]	6.4[c]	7.8[d]	

Source	SEm	CD (0.05)	CV%
Treatment (T)	0.592	1.71	40.77
Storage time (P)	1.026	2.95	
T x P	1.451	NS	

Data presented as means ± SD (n = 3).
SEm: Standard error of mean; CD: Critical difference; CV: Coefficient of variance; T x P: Interaction effect
[a-d]: values with different letters within a column are significantly different at 5% level of significant (i.e. P < 0.05).

Ability of ferroin dye to detect presence of rancid ghee mixed with fresh ghee

The ferroin dye test was carried out for detection of rancid ghee mixed (40%) with fresh ghee. The ghee samples were also analyzed for peroxide value and flavor score. The average results obtained for this qualitative test are presented in table 3 and depicted in figure 1. On day 0, the peroxide values of the control and treated ghee samples were 0.2 and 1.5 meq O_2/kg fat, respectively; a slight difference in the ferroin dye test results was observed (Figure 3). A crimson red color was observed in the control whereas a light red color was observed in the treated (40% added rancid ghee) sample.

Table 2 : Changes in flavor score of ghee samples obtained upon storage at 80° ± 2 °C.

Treatments	Storage time (Days)						Mean
	0	2	4	6	8	10	
Control	9.0 ± 0.0	8.8 ± 0.11	8.3 ± 0.09	7.8 ± 0.15	7.5 ± 0.0	5.8 ± 0.08	7.8
Treated (40%)	7.7 ± 0.22b	7.2 ± 0.14	7.0 ± 0.08	6.5 ± 0.06	5.8 ± 0.12	3.9 ± 0.17	6.3
Means	8.3[a]	8.0[b]	7.7[c]	7.2[d]	6.6[e]	4.9[f]	-

Source	SEm	CD (0.05)	CV%
Treatment (T)	0.050	0.1	6.13
Storage time (P)	0.087	0.25	
T x P	0.123	NS	

Data presented as means ± SD (n = 3).
SEm: Standard error of mean; CD: Critical difference; CV: Coefficient of variance; T x P: Interaction effect
[a-f]: values with different letters within a column are significantly different at 5% level of significant (i.e. P < 0.05).

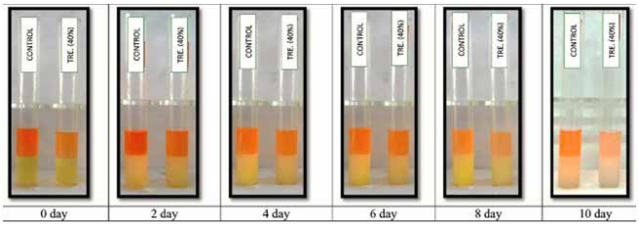

Figure 1: Comparison of control and treated (40% rancid) ghee samples in development of color using Ferroin test during storage at 80 ± 2 °C.

Upon storage, the peroxide values increased and the ferroin test color changed in both samples. On day 2 of storage, clear ferroin test color differences were observed in both samples of ghee. In the control, the color became crimson red whereas light red color was noticed in treated ghee samples. On the same day, the peroxide values were 1.3 and 2.7 meq O_2/kg fat for control and treated samples, respectively and the corresponding flavor scores were 8.8 and 7.2. On further storage, clear color differences were observed in both samples. At the end of storage, pale cream and yellowish-white colors were observed in the control and treated samples, respectively. Clear differences were observed between the control and treated ghee samples in the ferroin dye test throughout the storage period. It appears that no previous attempts have been made to use the ferroin dye test to detect mixing of rancid ghee with fresh ghee, or even to use such dye test to detect mixing of rancid oils/fats in other fats and oils.

Optimization of type and amount of solvent used in the ferroin test

Optimization of solvent

Methanol, chloroform and a chloroform: methanol mixture (7:3) were used to dissolve 5 mL ghee samples. Five mL of each solvents were used to dissolve both the ghee samples i.e. control (fresh ghee) and treated (40% rancid ghee

Table 3: Development of colors in ferroin dye test during storage of ghee at 80 ± 2 °C.

Storage period (Days)	Peroxide value (meq O_2/kg fat)		Flavor score		Ferroin Dye Test	
	Control	Treated (40%)	Control	Treated (40%)	Control	Treated (40%)
0	0.2 ± 0.01	1.5 ± 0.09	9.0 ± 0.0	7.7 ± 0.22	Crimson Red	Light red
2	1.3 ± 0.02	2.7 ± 0.06	8.8 ± 0.11	7.2 ± 0.14	Crimson Red	Light red
4	1.3 ± 0.03	3.5 ± 0.17	8.3 ± 0.09	7.0 ± 0.08	Light red	Orange
6	3.8 ± 0.22	5.0 ± 0.07	7.8 ± 0.15	6.5 ± 0.06	Light red	Yellowish white
8	3.4 ± 0.26	9.5 ± 0.70	7.5 ± 0.0	5.8 ± 0.12	Pale cream	Yellowish white
10	4.7 ± 0.56	11.0 ± 0.86	5.8 ± 0.08	3.9 ± 0.17	Pale cream	Yellowish white

Data presented as means ± SD (n = 3).

mixed with fresh ghee). The ferroin dye was added and color development was observed. Three replications were performed. The results are shown in figure 2. When the ghee samples were dissolved in methanol, there was a clear differentiation between the control and treated samples: the control samples showed a crimson red color whereas the treated sample showed a light red color. When the ghee was dissolved in chloroform or chloroform-methanol (7:3), there was no clear color differentiation between the control and treated samples. Methanol was therefore considered the best solvent and was used for optimization of the solvent quantity.

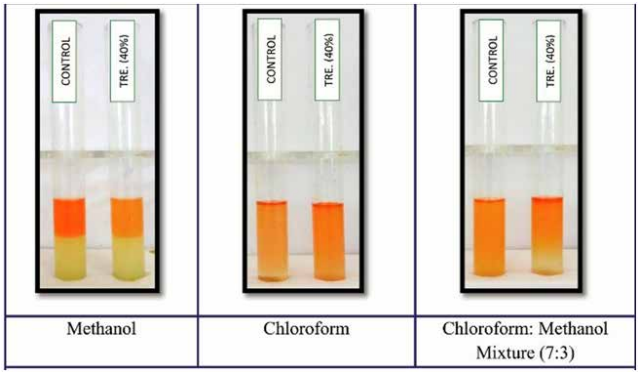

Figure 2: Optimization of type of solvent used for ferroin test.

Optimization of amount of methanol

Various quantities of 2, 5, 8 and 10 mL, of methanol were evaluated. Three replications were performed. The results are shown in figure 3. When the ghee samples were dissolved in 5 mL of methanol, there was a cleared differentiation between the control and treated samples; the control samples showed a crimson red color whereas the treated sample showed a light red color. When the samples were dissolved in the 2, 8, 10 mL of methanol there was no clear color differentiation between control and treated samples. Five mL was considered the optimal volume of methanol to use in the test and was used in further analyses.

Optimization of amount of ghee used in the ferroin test

Various quantities, 2, 5, 8 and 10 mL, of ghee samples were taken for optimization. Three replications were performed, and the results are shown in figure 4. The 5 mL sample showed clear color differentiation; the control samples showed a

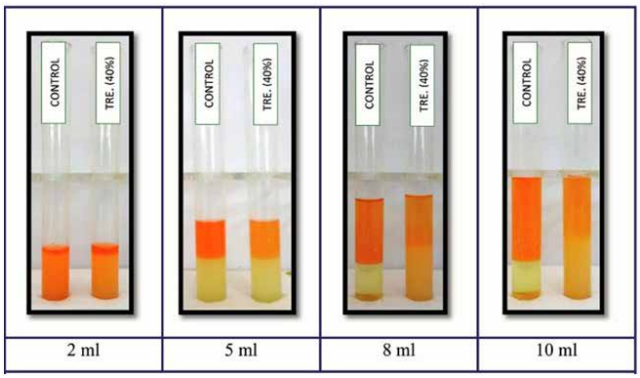

Figure 3: Optimization of amount of methanol used for ferroin test.

crimson red color whereas the treated sample showed light red color. When. 2, 8, 10 mL samples were used, there was no clear color differentiation between the control and treated samples. Therefore, the quantity of 5 mL ghee was considered optimal and was used in further analyses.

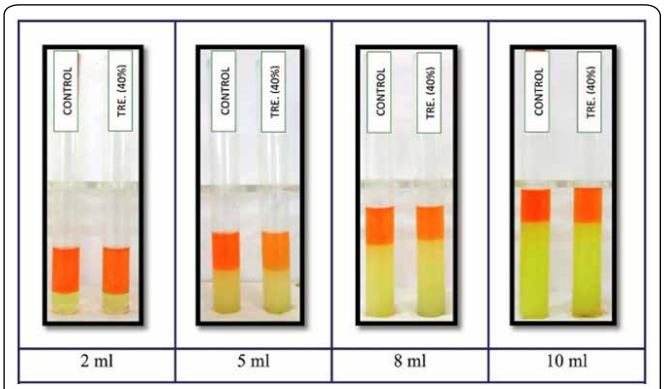

Figure 4: Optimization of amount of ghee used for ferroin test.

Optimization of temperature and duration of heating in ferroin test

Optimization of temperatures of heating

The test was conducted at 37, 45 and 55 °C. The duration of heating was kept constant at 10 min. Three replications were performed, and the results of color development are shown in figure 5. When temperature of heating was 55 °C, there was clear color difference: the control samples showed crimson red color whereas the treated sample showed a light red color. Heating

at 37 and 45 °C did not produce a clear color differentiation between the control and treated samples. Therefore, 55 °C was considered optimal and used in further analyses.

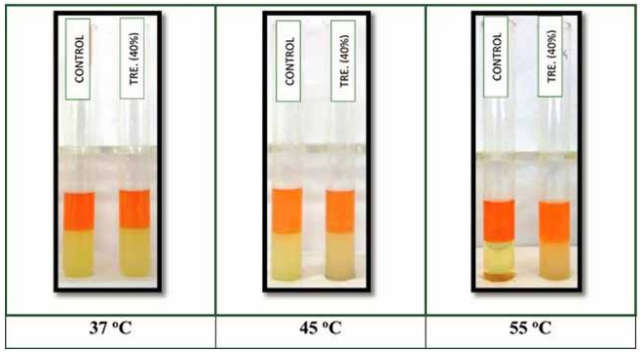

Figure 5: Optimization of temperature used for ferroin test.

Optimization of duration of heating

The ferroin dye were performed at 55 °C for 5, 10 and 15 min. Three replications were performed, and the results are shown in figure 6. Heating for 15 min resulted in clear color formation; the control samples showed a crimson red color whereas the treated sample showed a light red color. Heating for 5 and 10 min produced no clear color differentiation between the control and treated samples. Therefore, heating at 55 °C for 15 minutes was considered optimal since it clearly differentiated between the control and treated sample and it was used in further analyses.

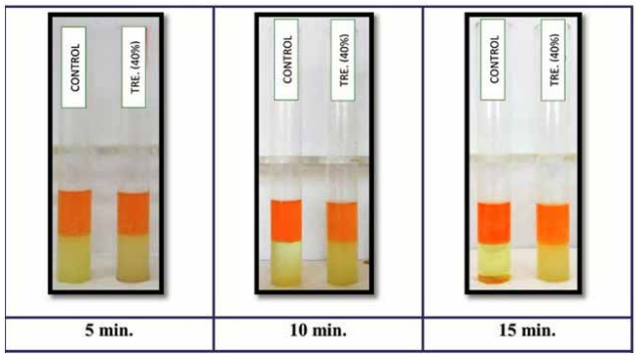

Figure 6: Optimization of duration for 55 °C temperature used for ferroin test.

Limit of detection of the ferroin test for rancid ghee in fresh ghee

Samples containing 10, 20, 30 and 40%, v/v, of rancid ghee in fresh ghee were subjected to the optimized ferroin test. All samples were kept at 80 ± 2 °C in a hot air oven. At 48 hour intervals, the samples were subjected to the ferroin test and the color formation observed. Storage continued until the flavor score was <6 and/or peroxide value of control sample was> 3.5 meq O_2/kg fat. A similar approach was used by Chauhan et al. to determine the LOD of urea in milk using p-dimethylaminobenzaldehyde [15].

Rancid ghee (10%) mixed with fresh ghee

The changes in peroxide value and flavor score as well as formation of color in the ferroin test for the control and 10% rancid sample are shown in table 4 and figure 7. As storage period increased, the peroxide values increased, and flavor scores decreased.

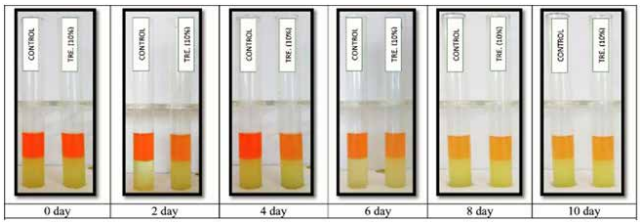

Figure 7: Ferroin test for T1 (10% - treated samples) compare with control samples.

On day 0, the ferroin test color was crimson red in the control samples and slightly light red in the treated (10% rancid) ghee sample. Upon further storage from day 2 to day 6, the ferroin test color changed from crimson red to pale orange in the control ghee samples and from light red to pale yellow in the treated ghee samples. At the end of storage i.e. after 10 days, the control samples produced a pale cream color and the treated sample a light pale cream color. In either case, there was a clear difference observed between the control and treated samples throughout the storage period. Hence the ferroin test can easily detect the presence of rancid ghee when mixed with fresh ghee at 10% level.

Rancid ghee (20%) mixed with fresh ghee

The changes in peroxide value and flavor scores as well as formation of color in ferroin test when 20% rancid ghee mixed with fresh ghee as show in table 4 and depicted in figure 8.

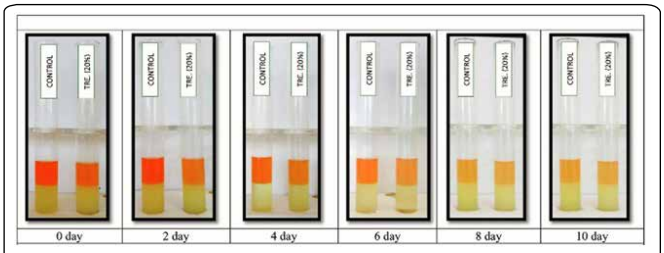

Figure 8: Ferroin test for T2 (20% - treated samples) compare with control samples.

It is observed from the table 4 and figure 8 that, on 0th days the color was crimson red in control ghee samples while orange color was obtained in the treated (20% rancid) ghee sample. Upon further storage from 2nd to 6th days, the color was changed from crimson red to pale orange in control ghee samples whereas from orange to pale cream was clearly found in treated ghee samples. At the end of storage i.e. on 10th days, the control has pale cream and light pale cream color was noticed for treated ghee samples. In either of cases, there were clear difference observed between control and treated samples throughout the storage period. Hence the ferroin test can easily detect the presence of rancid ghee when mixed with fresh ghee at 20% level.

Rancid ghee (30%) mixed with fresh ghee

The changes in peroxide value and flavor scores as well as formation of color in ferroin test when 30% rancid ghee mixed with fresh ghee as show in table 4 and depicted in figure 9.

Table 4: Changes in parameters of treated ghee samples measured by ferroin test.

Storage period (Days)	Peroxide value (meq O_2/kg fat)					Flavor score					Ferroin Dye Test				
	Control	T1 (10%)	T2 (20%)	T3 (30%)	T4 (40%)	Control	T1 (10%)	T2 (20%)	T3 (30%)	T4 (40%)	Control	T1 (10%)	T2 (20%)	T3 (30%)	T4 (40%)
0	0.2 ± 0.03	0.5 ± 0.10	0.9 ± 0.0	1.3 ± 0.03	1.8 ± 0.15	9.0 ± 0.0	8.0 ± 0.0	7.1 ± 0.0	6.4 ± 0.17	5.5 ± 0.17	Crimson red	Slightly Light red	Orange	Pale orange	Pale orange
2	0.6 ± 0.07	1.1 ± 0.10	1.4 ± 0.09	1.9 ± 0.03	3.3 ± 0.84	8.4 ± 0.29	7.4 ± 0.17	6.4 ± 0.17	5.4 ± 0.0	4.4 ± 0.0	Crimson red	Light red	Orange	Pale orange	Pale orange
4	1.5 ± 0.21	1.6 ± 0.23	2.1 ± 0.0	3.4 ± 0.03	4.4 ± 0.64	7.1 ± 0.17	6.1 ± 0.17	5.1 ± 0.0	4.0 ± 0.0	3.0 ± 0.0	Light red	Orange	Pale orange	Pale orange	Pale orange
6	2.3 ± 0.15	2.5 ± 0.20	2.9 ± 0.13	3.7 ± 0.06	5.8 ± 1.47	6.1 ± 0.17	5.0 ± 0.0	4.1 ± 0.13	3.1 ± 0.29	2.3 ± 0.0	Pale orange	Pale yellow	Pale cream	Pale cream	Pale cream
8	3.0 ± 0.21	3.2 ± 0.17	3.5 ± 0.35	4.7 ± 0.62	8.2 ± 1.59	5.4 ± 0.29	4.1 ± 0.0	3.4 ± 0.17	2.6 ± 0.29	1.8 ± 0.33	Sand yellow	Pale cream	Pale cream	Pale cream	Pale cream
10	4.1 ± 0.26	4.5 ± 0.30	5.6 ± 0.07	7.5 ± 0.23	11.0 ± 0.73	4.0 ± 0.0	3.0 ± 0.17	1.9 ± 0.33	1.0 ± 0.0	1.0 ± 0.0	Pale cream	Light pale cream	Light pale cream	Light pale cream	Light pale cream

Data presented as means ± SD (n = 3).

From the table 4, it is observed that as storage period increases, the peroxide value increases, and flavor scores were decreases.

It is observed from the table 4 and figure 9 that, on 0th days the color was crimson red in control ghee samples while pale orange color was obtained in the treated (30% rancid) ghee sample. Upon further storage from 2nd to 6th days, the color was changed from crimson red to pale orange in control ghee samples whereas from pale orange to pale cream was clearly found in treated ghee samples. At the end of storage i.e. on 10th days, the control has pale cream and light pale cream color was noticed for treated ghee samples. In either of cases, there were clear difference observed between control and treated samples throughout the storage period. Hence the ferroin test can easily detect the presence of rancid ghee when mixed with fresh ghee at 30% level.

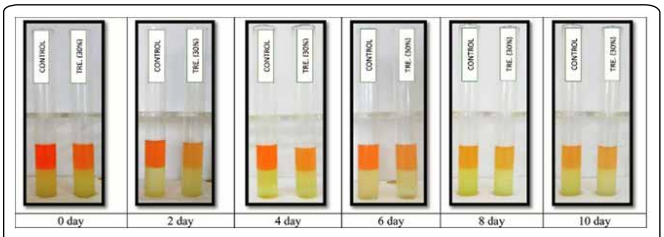

Figure 9: Ferroin test for T3 (30% - treated samples) compare with control samples.

Rancid ghee (40%) mixed with fresh ghee

The changes in peroxide value and flavor scores as well as formation of color in ferroin test when 40% rancid ghee mixed with fresh ghee as show in table 4 and depicted in figure 10.

From the table 4, it is observed that as storage period increases, the peroxide value increases, and flavor scores were decreases.

It is observed from the table 4 and figure 10 that, on 0th days the color was crimson red in control ghee samples while pale orange color was obtained in the treated (40% rancid) ghee sample. Upon further storage from 2nd to 6th days, the color was changed from crimson red to pale orange in control ghee samples whereas from pale orange to pale cream was clearly found in treated ghee samples. At the end of storage i.e. on 10th days, the control has pale cream and light pale cream color was noticed for treated ghee samples. In either of cases, there were clear difference observed between control and treated samples throughout the storage period. Hence the ferroin test can easily detect the presence of rancid ghee when mixed with fresh ghee at 40% level.

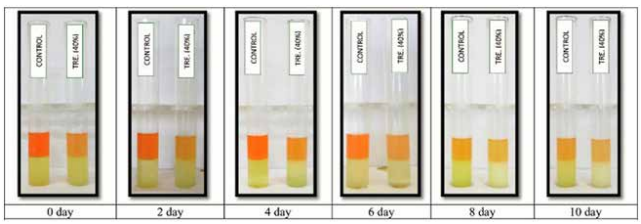

Figure 10: Ferroin test for T4 (40% - treated samples) compare with control samples.

The ferroin test can be used for detection of rancid ghee mixed with fresh ghee. The color chart showing different colored which differentiate between fresh ghee and rancid ghee samples is shown in figure 11.

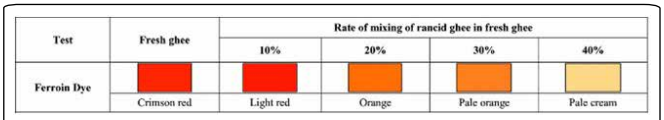

Figure 11: Colour chart for detection of rancid ghee mixed in fresh ghee.

Conclusions

Oxidative deterioration of ghee affects not only its economic value but also its appetizing flavor, reducing its

palatability. The disposal of rancid and poor-quality ghee is a problem for producers who are tempted to dispose such rancid ghee by partly replacing good quality ghee with rancid ghee. The developed qualitative ferroin dye test will be useful for quality control personnel for the rapid detection of rancid ghee mixed with fresh ghee.

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Conflicts of Interest

The authors report no conflicts of interest.

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