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Detection of Dry Milk in Pasteurized Milk and Yoghurt*

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Abstract. Two methods were developed to detect dried milk in fresh milk and yoghurt. The first method was based on the reaction with thiobarbeturic acid (TBA) while the second was based on measuring the milk reducing substances (MRS) with ferricyanide. The simple correlation coefficients (r) between percentage dried milk and TBA ($r=0.9861$), and MRS ($r=0.9994$) values were highly significant ($P \leq 0.01$). Therefore, either TBA or MRS methods could be used to detect dried milk in fresh pasteurized milk and yoghurt. However, MRS method could better detect low levels of added NFDM to fresh pasteurized milk and yoghurt.

Introduction

The lower prices of skim milk powder as compared to fresh milk makes it financially attractive for factories to adulterate fresh milk with skim milk powder in Saudi Arabia. Recombined milk products represented about 50% of the total milk manufactured in Saudi Arabia in 1987 [1]. Sterilized milk was made exclusively from recombined milk and constituted 70% of the total processed fluid milk in the central province of Saudi Arabia in 1984 [2], while more than 95% of sterilized milk was made from recombined milk in 1987 [3]. Consumers in Saudi Arabia prefer fresh milk [4] and since recombined milk is cheaper than fresh milk as a result of subsidizing milk powder, there is a possibility of adulteration of fresh milk with recombined milk. Therefore, a quick method to detect milk powder in fresh milk products is necessary for the quality control of milk products and help Saudi Arabian Standards Organization (SASO) to establish such methods.

When milk is subjected to prolonged heating, many complex chemical changes take place. Two of these changes which are of particular interest are the browning

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reaction and the liberation of reducing substances. Various methods have been investigated for quantitative determination of skim milk powder in fresh milk. Cardwell and Herzer [5] described a colorimetric method for the determination of NFDM in fresh milk. They found that the linear relationship existed between the protein reducing substances (PRS) readings and NFDM content in fresh milk, but the several methods which measure browning color in milk have been developed also. Enzymatic extraction techniques such as proposed by Patton [6], water-alkaline extraction techniques suggested by Jenness and Coulter [7], or digestion of the intermediate amino-sugar complex compounds by oxalic acid and then reacting the filtrate with thiobarbituric acid (TBA) as recommended by Keeney and Bassette [8] have been used.

The objective of this study was to develop a simple and quick method to detect and measure milk powder level in fresh milk products such as yoghurt and pasteurized milk. These methods are based on the thiobarbituric acid (TBA) and milk reducing substances (MRS) of milk.

Materials and Methods

Preparation of milk samples

Fresh milk was obtained from King Saud University dairy farm. The milk was homogenized and pasteurized at 73°C for 15 sec. Non-fat dry milk (NFDM) was obtained from New Zealand Dairy Cooperative. Milk samples were prepared by mixing fresh pasteurized milk with reconstituted NFDM (10 gm NFDM were dispersed in 100 ml distilled water) to have concentrations of 10, 20 and 30% of fresh pasteurized milk with NFDM.

Yoghurt preparation

Yoghurt culture (combined *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* CH-1) was obtained from Chr. Hansen Laboratory Inc., Milwaukee, W1. The culture was prepared by inoculation at a 2% level into fresh pasteurized milk containing 15 or 30% reconstitu ed NFDM. Yoghurt was manufactured as outlined by Kosikowski [9].

Detection of dried milk in fluid milk and yoghurt

The two methods developed by Abu-Lehia [10] were used to detect dried milk in fluid milk and yoghurt.

TBA method

The milk and yoghurt samples were tempered to room temperature (21°C). To a 50 ml tube a 10 ml aliquote of each tempered milk or yoghurt samples was added, followed by addition of 5 ml of oxalic acid solution (0.3 N). Then, the tubes were placed in a water bath at 95°C for exactly 1 hr. Tubes were removed from the water bath and immediately covered with parafilm and aluminum foil and cooled to 38°C. Five ml of TCA (40%) were added to each tube and mixed well. Whatman # 42 filter paper was used to filter the reaction mixture, then 4.0 ml of each filtrate was transferred to a clean tube and 1 ml of 0.05 M thiobarbituric acid (TBA) was added and mixed well. The tubes were heated to 40°C for exactly 35 min in a water bath. The tubes were cooled in an ice water bath, and spectrophotometer 20 (Bausch and Lomb) at 443 nm was used for the determination of light transmittance. A blank consisting of distilled water, oxalic acid solution, TCA and TBA was run along with and in the exact same manner as the tested samples.

MRS method

Milk and yoghurt samples were prepared and tempered as described above. A 1 ml aliquote of the sample was transferred to a 25 ml test tube. To each tube, 5 ml of urea solution (10 M) was added and contents were mixed by super-mixer. Three ml of potassium-ferricynide solution (0.12%) were added to the tube contents and mixed by super-mixer. Tubes were heated in a water bath at 70°C for exactly 40 min, then cooled to below 32°C. Two ml of ammonium sulfate solution (10 M) was added to the contents of the tubes and mixed by super-mixer. Then, 0.4 ml of 40% TCA was added to the tube and mixed well. Mixture was then filtered through Whatman # 40 filter paper. Aliquote of each filtrate (2.5 ml) was transferred to a spectrophotometric tube and diluted with 2.5 ml of distilled water and mixed well with super-mixer. Tubes were immediately placed in water bath maintained at 32.3°C for 5 min. A 0.5 ml aliquote of a 0.2% ferric chloride solution was added. Reaction was allowed to proceed for exactly 30 min. Percent transmittance was read on spectrophotometer (Bausch and Lomb) at 610 nm. A blank consisting of distilled water, urea, potassium ferricyanide, ammonium sulfate and ferric chloride was run along with and in the exact same manner as the test samples.

Statistical analysis

All determinations were carried out in triplicate. Two-way analysis of variance followed by separation of means was carried out according to the Duncan's new multiple range test. Correlation coefficient (r) and linear regression equation as well as multiple regression equation were used to examine the relationship between NFDM content in milk or yoghurt and TBA or MRS values [11].

Results and Discussion

This study involved the development of two methods to detect reconstituted NFDM in the milk products namely pasteurized milk and yoghurt. The first method was based on the TBA reaction. This technique was based mainly on that of Keeney and Bassette [8] with slight modification. Browning in milk is caused by a maillard-type interaction between the free amino groups of milk proteins and the aldehyde group of lactose [12]. It is a chemical change that takes place in milk when milk is subjected to prolonged heating.

The results shown in Table 1 and 2 indicate that TBA values increased as the level of NFDM solids added to milk and yoghurt increased. The differences among means were significant ($P \leq 0.05$). However, the use of TBA to detect reconstituted NFDM in fluid milk is limited when the amount added is small (NFDM≤15%, data not shown). Acidity level of yoghurt also had an effect on TBA values (Table 2). TBA values increased as the acidity of the acidified milk and the yoghurt increased. The incease in TBA could be due to the aldehydes which are formed when acidity increases. Also, data in Table 1 and 2 show that TBA values were higher in yoghurt compared to pasteurized milk. This could be due to the heat treatment of the milk which is used for yoghurt manufacturing. The regression analysis of the data pre-

Table 1. TBA readings of milk containing different level of reconstituted milk powder (%)

	Reconstituted milk powders (%) in milk				
	0.0	**10**	**20**	**30**	**100**
O.D.	0.068^{e}	0.076^{d}	0.085^{c}	0.10^{b}	0.15^{a*}

*Means not followed by the same letter are significantly different ($P \leq 0.05$).

Table 2. TBA readings of acidified milk and yoghurt containing different percentage of reconstituted powder at different levels of acidity (O.D.)*

Acidity	Reconstituted milk powder (%) in yoghurt milk		
	0.0	**15**	**30**
0.20±0.01	0.167^{g}±0.001	0.182^{e} ±0.002	0.194^{d}±0.003
0.32±0.01	0.184^{e}±0.002	$0.197^{c,d}$±0.004	0.206^{c}±0.009
0.64±0.03	0.210^{c}±0.003	0.221^{b} ±0.003	0.230^{a}±0.008

*Means not followed by the same letter are significantly different ($P \leq 0.05$).

sented in Table 1 indicates that the simple correlation coefficient (r=0.9861) between % reconstituted NFDM in milk samples and TBA reaction was highly significant ($P \leq 0.01$) as shown graphically in Fig. 1. The equation which describes the relation between percentage reconstituted NFDM and TBA reaction was $y=0.069+(0.0008)\times$. These results agree with those reported by Abu-Lehia [10]. In addition, a multiple regression analysis for yoghurt experiment data were used to study the relationships between %NFDM, %T.A. and TBA values (optical density, O.D. readings). These relationships were described in the following equation, $y=0.154+0.0008\ x_1+0.087x_2$ where y=O.D. values x_1=%NFDM and x_2=%T.A. Therefore, TBA value could be used to detect reconstituted NFDM in both fluid milk and yoghurt especially when added at high levels.

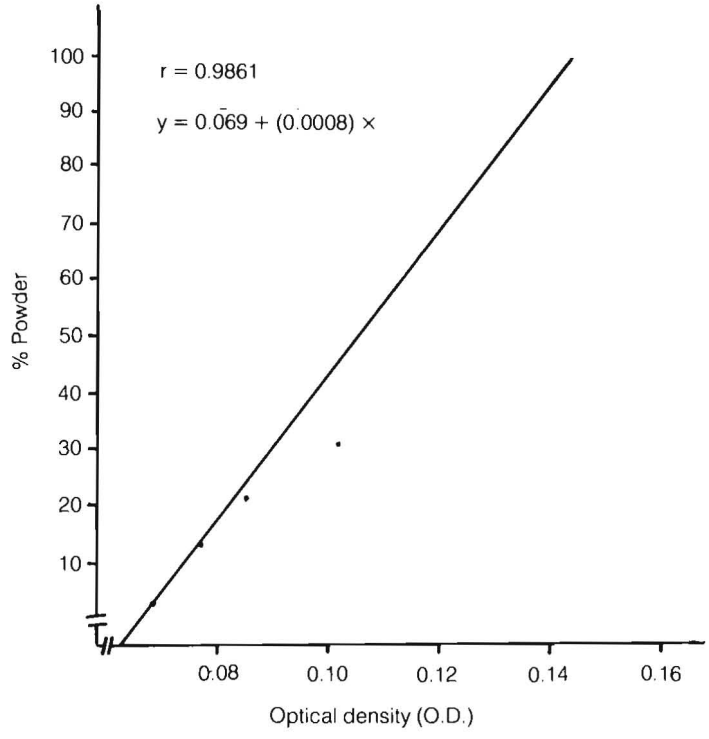

Fig. 1. Relationship between %NFDM and TBA reaction.

Data presented in Table 3 indicate the MRS values increased significantly ($P \leq 0.05$) as the level of reconstituted NFDM solids added to milk increased. These findings agree with those reported by Abu-Lehia [10]. There were significant differences ($P \leq 0.05$) among means. Moreover, MRS values of acidified milk or yoghurt were increased as the acidity level increased (Table 4). The major components of milk serum are lactose and serum proteins which are responsible for reduction of potassium-ferricyanide. In addition, other components such as amino-sugar complex compounds which may have developed as a result of the drying process and storage conditions could reduce potassium-ferricyanide. So the source of high reducing substance value in the reconstituted dried whey is coming not only from lactose and whey protein, but also from amino-sugar complex compounds. This is also true for yoghurt, however, the effect of acidity on MRS values in yoghurt could be due also to the effect of (H^+) concentration on the potential oxidation reduction in yoghurt (Table 4). To clarify this point, yoghurt was made by direct acidification and results obtained (not shown) for MRS values has almost the same values as those obtained from yoghurt made using a starter culture. This indicates that reducing substances are coming from the milk components.

Table 3. MRS readings of milk containing different levels of reconstituted milk powder (%)*

	Reconstituted milk powder (%) in milk				
	0.0	**10**	**20**	**30**	**100**
O.D.	0.0335^{e} ±0.005	0.0915^{d} ±0.008	0.1487^{c} ±0.015	0.1962^{b} ±0.018	0.538^{a} ±0.021

*Means not followed by the same letter are significantly different ($P \leq 0.05$).

Table 4. MRS of acidified milk and yoghurt containing different percentage of reconstituted milk powder at different levels of acidity*

Acidity	Reconstituted milk powder (%) in yoghurt milk		
	0.0	**15**	**30**
0.20±0.01	0.080^{i}±0.003	0.162^{g}±0.007	0.335^{d}±0.011
0.32±0.02	0.095^{h}±0.008	0.185^{f}±0.010	0.623^{b}±0.012
0.75±0.04	0.253^{c}±0.009	0.514^{c}±0.012	0.975^{a}±0.018

*Means not followed by the same letter are significantly different ($P \leq 0.05$).

The regression analysis of the data presented in Table 3 indicates the relationship between MRS values and % NFDM in fresh milk as shown graphically in Figure 2. The correlation coefficient (r) between MRS values and % NFDM was highly sig-

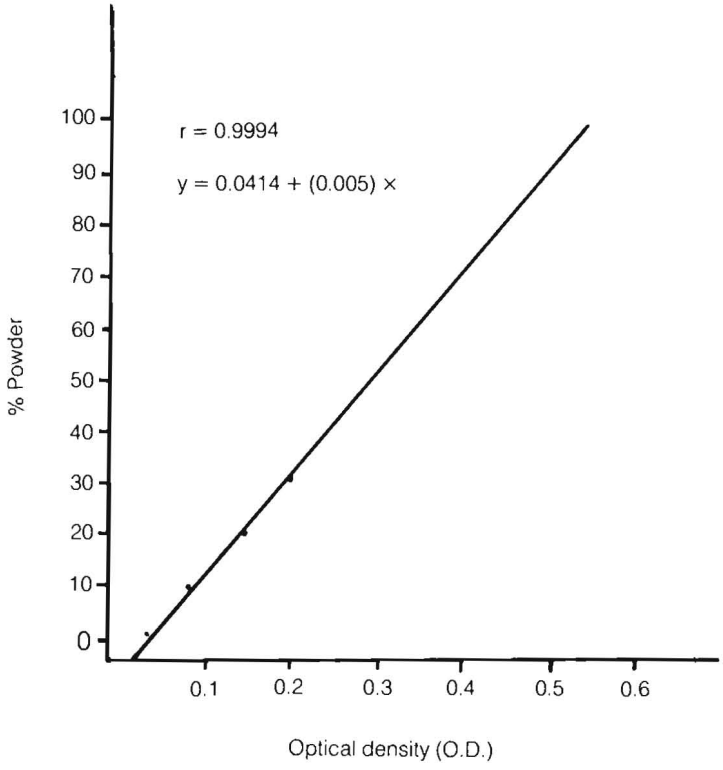

Fig. 2. Relationship between %NFDM and MRS reaction.

nificant ($P \leq 0.01$) as 0.9994 and the regression linear equation to describe this relation was $y=0.0414+(0.005)x$. These results indicate that MRS values are very highly associated with NFDM levels. For yoghurt experiment data, a multiple regression equation was used to test the relationships between %NFDM, %T.A. and MRS readings (O.D. values) as shown in the following equation $y=0.185+0.0167x_1+0.691x_2$ where y=MRS readings (O.D.), x_1 and x_2 = %T.A. It was found that %NFDM and %T.A. in yoghurt or acidified milk were well associated with MRS readings ($P \leq 0.001$). Therefore, the method was capable of differentiating between low levels of NFDM in fresh milk and yoghurt samples studied. These data agree with those reported by Cardwell and Herzer [5] using PRS method

in measuring added NFDM to milk. MRS method described in this paper depends mainly on the dispersion of dairy products with urea solution (10 M) to impose buried reducing substances to reduce potassium-ferricyanide during heating process, after which the precipitation of protein was accomplished by a technique using precipitating agent (both TCA and ammonium sulfate, not any).

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الكشف عن الحليب المجفف في الحليب المبستر واليوغرت

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ملخص البحث. تم تطوير طريقتين للكشف عن الحليب المجفف في الحليب الطازج واليوغرت. وتعتمد الطريقة الأولى على تفاعل حمض الثيوباربيتوريك مع نواتج التفاعل البني من تسخين الحليب أما الطريقة الثانية فتعتمد على تقدير المواد المختزلة في الحليب. ولقد كان معامل الارتباط بين النسبة المئوية للحليب المجفف ونواتج التفاعل البني معنويًا بدرجة عالية ($r = 0.9861$) وكذلك وجد معامل الارتباط بينها وبين المواد المختزلة في الحليب ($r = 0.9994$) مهمًا من الناحية الإحصائية ($P < 0.01$). لذا يمكن استخدام أي من الطريقتين للكشف عن الحليب المجفف في الحليب الطازج والمحمض واليوغرت، إلا أن طريقة المواد المختزلة في الحليب بإمكانها الكشف بدرجة أدق عن المستويات المنخفضة من إضافة الحليب المجفف إلى الحليب الطازج المبستر واليوغرت.