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Physico-chemical, Microbiological Parameters and Adulteration in Processed Dairy Products in Pakistan

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Abstract

Adulteration of dairy products with chemicals such as Caustic soda, Urea, Antibiotics and Microbiological contamination (high Total plate count, Coliform count and *S. aureus*) in processed dairy products samples constitute a potential public health hazard. A study was carried out to determine the microbiological quality and adulteration in various processed dairy products from various brands prevalent in the market.

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Samples of different dairy products including: (i) Yogurt, (ii) Cheese, (iii) Butter, (iv) Ice cream and v) Milk powder were collected from various places and processed in the laboratory for physic-chemical, microbiological and adultration analysis was carried out. (i)Yogurt samples from different Brands showed non-significant ($P>0.05$) difference in Total Plate Count (TPC), Coliform Count (CC) whereas significant ($P<0.05$) difference was observed in *Streptococcus aureus* Count (SAC), (ii) Cheese samples showed non-significant ($P>0.05$) difference in TPC whereas significant ($P<0.05$) difference was observed in SAC, (iii) Butter samples showed significant ($P<0.05$) difference in TPC, CC and SAC, (iv) Ice cream samples showed significant ($P<0.05$) difference in TPC, CC and SAC; and (v) Milk powder from various brands showed non-significant ($P>0.05$) difference in CC and SAC. All dairy products were negative for chemical adulterants tests i.e. urea, formaldehyde, neutralizers, starch, boric acid, quaternary ammonium compounds, while H2O2 was slightly positive in yogurt, milk powder and Ice cream samples of different brands. It is concluded that the dairy product in the market are safe from adulterants. Further butter and ice cream are showing a significant TPC, CC, and SAC.

***Key words*:** Physico-chemical quality; Microbial quality; chemical adulterants; dairy products.

1. Introduction

In 2002 Pakistan was producing 32 million tons of milk that was slightly rise than Germany [5]. There is a great potential for dairy industry but the sector operates mostly in the informal economy and needs a consistent effort to formalize and be able to contribute better to the national economy. There are hardly 15 milk processing plant (mainly UHT fluid milk, milk powder and yogurt in Pakistan). Only about 3 percent milk is being processed and 97 percent is consumed as raw milk [14] .

Milk and dairy products are one of the most important food products that enjoy special significance in terms of its various nutritional properties such as protein, lactose, fat, minerals and vitamins. Many studies have been made on its constituents and physicochemical characteristics [21].

Milk and dairy products form a significant part of the diet in many countries and a substantial part of food expenditure goes on milk and dairy products. Basic public health and hygienic considerations require that consumer be provided with safe and pure milk that is hygienically clean and unadulterated. Suppliers of milk and milk in Pakistan dairy industry appear to have found three ways to increase their margin from the sale of milk, 1) Addition of water 2) Removal of valuable components i.e. milk fat 3) A combination of one and two with the addition of additives, such as starch, to increase the total solids [20]. Sometime adulteration of milk is made by addition of chemicals i.e. caustic soda, urea and antibiotics which can cause serious health problem such as cancer, diabetes and kidney failure [7] and ultimately affect the health of our nation.

Adulteration of milk and dairy products is one of the most serious issues in the dairy industry and causes economic losses and major health problems to consumers. Due to the limited numbers of large dairy farms, milk handling processes in the traditional system are unhygienic and there is insufficient enforcement of standards, resulting in poor quality milk products. In order to keep milk safe middleman adds ice to the milk. In addition,

microbiological contamination occurs due to addition of ice in the milk [2]. The middleman increases the milk quantity by adding water, vegetable oil, whey powder and other ingredients to increase the solids of the milk. Antibiotics and Hydrogen peroxide are often used as preservatives [5].

The adulterants in milk include water, starch, vegetable oil, whey powder and hazardous substances such as antibiotics, caustic soda, urea, formalin, detergents and other chemical preservatives. Adulteration in milk and dairy products is a serious public health hazard and is a very serious problem in Pakistan. Keeping in view these facts, the present study was planned with the objectives to study the microbiological quality of dairy products and to determine the chemical adulterants and residues in dairy products.

2. Materials and methods

2.1 Collection of samples

The dairy product samples were collected from the market and then physicochemical, microbiological, and adulteration analyses were performed at Quality Control Laboratory, University of Veterinary and Animal Sciences (UVAS), Lahore. Three samples of each brand were collected from local market Lahore.

Yoghurt samples from Haleeb and Nestle brands, Cheese samples from Nurpur Dairies, Haleeb and Adams, Butter samples from Gourmet, Haleeb and Nestle, milk powder samples from two different brands namely, Nurpur Dairies and Haleeb whereas, Ice cream samples were collected of three different brands namely, Gourmet, Walls and Yummy of cup types.

2.2 Sampling procedure

Dairy product samples were collected in clean sterilized containers and put in ice chest, whereas milk powder was collected in zip polythene bag. These samples were transported for analysis to Quality Control Laboratory, University of Veterinary and Animal Sciences, Lahore.

2.3 Sterilization

All glassware like pipette, test tubes, Petri dishes, beakers, and flasks were thoroughly cleaned and sterilized in an oven at 180^0C for 2 hours. All media and solutions were prepared in distilled water and autoclaved at 121^0C at 15 lb pressure for 15 minutes using the procedure of [3].

2.4 Microbiological tests

Dairy product samples were tested for *Total plate count*, *Coliform count*, *Staphylococcus aureus*, and Yeast and Mould count by the methods prescribed by [3].

2.5 Sample preparation

Prepared the dilution blanks by pipetting 9 ml of Phosphate Buffer Solution (PBS) into sterile test tubes. Dairy

product sample was taken with 1 ml sterilized pipette and added into first dilution blank, and then shake the test tube 25 times in 1-foot arc within 7 seconds and then 1ml is transferred to the next dilution blank. Continued the same procedures as the required dilution obtained according to procedure of [3].

2.6 Total plate count

The Total plate count (TPC) is intended to indicate the level of microorganisms in the product. Plate count agar media (Table1) was used for the determination of *Total Plate Count* in dairy product samples as described in [3].

2.7 Colony counting

Colonies were counted from the plates showing the colonies between 20-200 with the help of colony counter [3].

Viable bacterial count = Average number of colonies × dilution factor

2.8 Coliform count

Violet Red Bile Agar (VRB) media (Table 2) was used for determination of *Coliform* count in dairy product samples. Media was prepared by following the procedure of manufacturer (Oxoid) [3].

2.9 Staphylococcus aureus count

Baired Parker Agar media (Table 3) was used to determine the *Staphylococcus aureus count* in dairy product samples. Media was prepared according to (Oxoid) for preparation of plates were poured and the spread plate method was used as described in` [3].

2.10 Yeast and mould

Saborad Dextrose Agar media (Table 5) was used for determination of yeast and mould in dairy product samples. Media was prepared by following the procedure of manufacturer (Oxoid) [3].

2.11 Chemical Adulterants Detection Tests

2.11.1 Formaldehyde

Adulteration of Formaldehyde was determined by mixing 02 ml sample of dairy products with 90% solution of sulfuric acid. Development of yellow color at the junction of two layers indicated the presence of formaldehyde.

2.11.2 Boric acid

For the detection of Boric Acid adulteration, 5 ml dairy product samples were taken in a test tube and 1 ml of concentrated hydrochloric acid was added, contents of test tube were mixed well for 1 minute and a strip of

turmeric paper was dipped into the acidified dairy products and development of red color exhibited the presence of boric acid.

2.11.3 Hydrogen peroxide

Presence of hydrogen peroxide was detected by mixing 1% Valeduum Peroxide in 6% sulfuric acid solution. Appearance of pink color was the evidence of adulteration of dairy products with hydrogen peroxide.

2.11.4 Starch

Presence of starch in dairy products was determined by adding 1ml (1 %) iodine solution in 3 ml dairy product samples. Presence of blue color indicated the presence of starch.

2.11.5 Neutralizer (sodium carbonates, sodium bi carbonates and sodium hydroxide)

Adulteration of neutralizers was estimated by mixing 1 ml of various dairy products sample with 1 ml Rosalic acid solution (1 % in distilled water). Development of red color indicated the adulteration of dairy products with neutralizers.

2.11.6 Urea

Adulteration of urea was detected by adding 01 ml 1.60% Para Dimethyl Benzaldehyde prepared in concentrated Hydrochloric Acid. Development of yellow color indicated the adulteration of dairy products with urea.

2.11.7 Quaternary Ammonium Compounds

Five ml of dairy products were taken in a centrifuge tube, 5 ml of tetra chloroethane and 2 ml of lactic acid solution was added and test tube was stopped and shaken vigorously for One minute, 02 ml of standard sodium hydroxide solution was added and centrifugation was done at 3200 r.p.m. for 5 minutes. Remove the top layer by decantation. Pipette out 2 ml of aliquot of tetra chloroethane in a test tube. Add 0.5 ml of citric acid buffer and 0.2 ml of eosin solution. Cork the tube and shake vigorously. Observe the color. Pink or red color in the tetra chloroethane indicates presence of QAC.

Adulteration tests were performed by following the procedures of [6].

2.12 Statistical analysis

The data thus obtained were statistically analyzed to compare the parameters of study through analysis of variance technique [16] and the means were compared through LSD whereas the comparison between dried milk samples and yoghurt samples were done through student's t- test.

3. Results and discussion

3.1 Microbiological Tests

3.1.1 Total Plate Count

The highest recorded mean of Total plate count was (4.60±0.627) log cfu/g in yoghurt samples of Haleeb brand and lowest was (4.49±0.498) log cfu/g in Nestle brand. ANOVA showed non-significant (P>0.05) difference in Total plate count of yoghurt samples. These results are agreed with Lin and his colleagues. [13] who reported maximum increase (P<0.05) in Total plate counts that ranged from 6.0-7.0 to 7.5-9.1 log cfu/g. Salisu and his colleagues.[18] also reported the mean total aerobic plate count (TAPC) was 3.37 x 104 cfu/ml while the range was 2.66 x 104 ± 8.3 x 103 to 1.13 x 105 ± 1.17 x 105 cfu/ml of yoghurt. The highest recorded mean of Total plate count was (4.93±1.128) log cfu/g in cheese samples of Nurpur brand while, lowest was (3.31±0.10) log cfu/g observed in Haleeb brand. ANOVA showed non-significant (P>0.05) difference of Total plate count in cheese samples of different brands. Our results are in agreement with [15] and Suleiman [19] who found that the mean Total plate counts/g, $\log^{10}$, in the fresh U.S. and Canadian cheeses were 3.54 and 5.22, and total viable count 1.0×104 c.f.u/g respectively. The highest mean of Total plate count recorded was (5.39±0.524) log cfu/g in butter samples of Gourmet brand and lowest was (3.67±0.349) log cfu/g in Nestle brand. There was significant (P<0.05) difference in Total plate count of butter samples. These results are similar to the reported by [22] who found Aerobic plate counts that ranged from 1.7 to 3.5 log cfu/g in butter samples. The highest recorded mean of Total plate count was (5.76±0.69) log cfu/g in ice cream samples of Gourmet brand and lowest was (4.31±0.275) log cfu/g in Walls brand. ANOVA showed the significant (P<0.05) difference in Total plate count of ice cream samples of different brands Table 1 and 2.

3.1.2 Coliform Count

The highest mean of Coliform count was (3.85±0.442) log cfu/g in yoghurt samples of Haleeb brand and lowest was (3.28±0.07) log cfu/g observed in Nestle brand. ANOVA table showed non-significant (P>0.05) difference of Coliform count in yoghurt samples of different brands.

The results of present studies regarding Coliform count is not in line with [11] who did not report Coliform count in yoghurts made from fresh or frozen milks. Ifeanyi and his colleagues. *[9]* reported the coliform count in the yoghurt samples were high; 4.4 x 105 CFU/ml. The highest recorded mean of Coliform count was (5.28±0.759) log cfu/g in cheese samples of Nurpur brands and lowest (3.38±0.110) cfu/log g were observed in Haleeb brand. ANOVA showed significant (P<0.05) difference of Coliform count in cheese samples of different brands. Conversely to our results, [1] did not report Coliform count in cheese while Hussein and his colleagues. [8] find out the the coliform count was high in locally produced cheese log 3.34 compared to the imported one log 3.09. The highest mean of Coliform recorded was (4.53±0.732) log cfu/g in butter samples of Gourmet brand and lowest was (3.38±0.013) log cfu/g observed in Nestle brand. ANOVA showed the significant difference of Coliform count in butter samples of different brands.

The highest mean of Coliform recorded was (5.41±0.616) log cfu/g in ice cream samples of Gourmet brand and

lowest was (3.79±0.37) log cfu/g observed in Walls brand. Similar results were reported by [12] who manufactured three ice creams with flavors of vanilla, chocolate and strawberry and observed Coliform counts with the level of 1.7 to 2.4 log cfu/g. ANOVA showed significant ($P<0.05$) difference of Coliform count in Ice cream samples. The highest mean of Coliform recorded was (4.16±0.826) log cfu/g in milk powder samples of Nurpur brand and lowest was (3.95±0.873) log cfu/g observed in Haleeb brand. ANOVA showed non-significant ($P>0.05$) difference of Coliform count among milk powder samples of different brands (Table 3).

Table 1: Analysis of variance of microbiological tests in yoghurt samples of different brands

	Yoghurt				**Cheese**			
SOV	**Difference**	**Mean square**	**F**	**P value**	**difference**	**Mean square**	**F**	**P value**
TPC - b/w groups	1	1.493	4.680	0.059	2	2.540	2.830	0.136
Error	4	0.319			6	0.897		
Total	5				8			
Coliform -b/w groups	1	0.859	3.632	0.092	2	2.989	9.519	0.013
Error	4	0.236			6	0.314		
Total	5				8			
Staph aureus - b/w groups	1	5.154	10.511	0.010	2	0.806	8.182	0.019
Error	4	0.245			6	0.098		
Total	5				8			

3.1.3 Staphylococcus aureus count

The highest recorded mean of Staph aureus was (4.52±0.557) log cfu/g in yoghurt samples of Haleeb brand and lowest was (3.63±0.079) log cfu/g in Nestle brand. ANOVA revealed significant ($P<0.05$) difference of Staph aureus of yoghurt samples.

The highest mean of Staph aureus recorded was (4.64±1.132) log cfu/g in cheese samples of Nurpur brands and lowest was (3.61±0.16) log cfu/g in Haleeb brand.

ANOVA showed significant (P<0.05) difference of Staph. Aureus count in cheese samples of different brands. Present results are in agreement with [17] who reported S. aureus counts were 5.16 log cfu/g in control cheese samples.

The highest mean of Staph aureus recorded was (5.35±0.736) log cfu/g in butter samples of Gourmet brand and lowest was (3.60±0.167) log cfu/g in Nestle brand.

Table 2: Mean (±S.E) total plate count, coliform Staph aureus count in yoghurt samples of different brands

Sample		Total Plate Count			Coliform count			Staph aureus count		
		Means ± S.E	Min	Max	Means ± S.E	Min	Max	Means ± S.E	Min	Max
Haleeb	Yoghurt	4.60 ±0.627	4.14	5.32	3.85±0.442	3.54	4.36	4.52 ±0.557	3.88	4.86
Nestle	Yoghurt	4.49 ±0.498	4.17	5.07	3.28±0.070	3.22	3.36	3.63 ±0.079	3.54	3.69
Adams	Cheese	4.88 ±1.186	4.64	5.59	4.85±0.594	4.46	5.54	4.10 ±0.517	3.51	4.44
Haleeb	Cheese	3.31 ±0.100	3.94	3.41	3.38±0.110	3.27	3.49	3.61 ±0.16	3.51	3.71
Nurpur	Cheese	4.93 ±1.128	4.80	5.61	5.28±0.759	4.41	5.74	4.64 ±1.132	4.53	4.79

There was significant (P<0.05) difference of Staph. Aureus count of butter samples. The highest mean of Staph aureus recorded was (6.38±0.536) log cfu/g in ice cream samples of Gourmet brand and lowest was (3.90±0.048) log cfu/g in Walls brand. ANOVA revealed significant (P<0.05) difference of Staph. Aureus Count in ice cream samples.

The highest mean of Staph aureus recorded was (4.89±0.299) log cfu/g in milk powder samples of Nurpur brand and lowest value (4.43±0.425 log cfu/g) was observed in Haleeb brand. ANOVA revealed non-significant (P>0.05) difference of Staph. Aureus count among milk powder samples of different brands (Table 4).

3.1.4 Yeast and Mould Count

Yeast and Mold count in cheese samples of Nurpur brand was 2.5×10^4 /g and 4.3×10^3/g in Haleeb samples.

In case of Gourmet butter, Yeast and mould count was3.1×10^4/g, while samples of Nestle showed 3.7×10^3/g. [4] reported high Yeast count 10^6–10^7 cfu/g in yoghurt and cheese samples, while lower counts found in cream, butter and ice cream.

3.1.5 Chemical Adulterants Detection Tests

Haleeb and Nestle yoghurt brands were negative for chemical adulterants tests i.e. urea, formaldehyde, neutralizers, starch, boric acid, quaternary ammonium compounds, while H_2O_2 was detected slightly positive in both yoghurt samples of Haleeb and Nestle Table 5. Similarly all the chemical adulterants tests were negative for milk powder samples of Haleeb and Nurpur brands while slightly positive for H_2O_2.

Results about ice cream samples showed that all the ice cream samples were negative for chemical adulterants tests while slightly positive for H_2O_2. Our study agreed with [10] who reported similar adulterants in dairy products.

Table 3: Analysis of variance of microbiological tests in butter samples of different brands.

	Butter Samples				**Ice cream Samples**				**Milk powder**			
SOV	**Difference**	**Mean square**	**F**	**P value**	**Difference**	**Mean square**	**F**	**P value**	**Difference**	**Mean square**	**F**	**P value**
TPC - b/w groups	2	2.482	10.275	0.0115	2	2.073	6.338	0.033	1	1.297	3.22	0.147
Error	6	0.241			6	0.327			4	0.402		
Total	8				8				5			
Coliform -b/w groups	2	2.482	10.275	0.011	2	2.273	6.676	0.029	1	0.064	0.088	0.780
Error	6	0.241			6	0.340			4	0.723		
Total	8				8				5			
Staph aureus - b/w groups	2	2.317	11.408	0.009	2	4.712	15.667	0.004	1	0.326	2.419	0.195
Error	6	0.203			6	0.300			4	0.135		
Total	8				8				5			

Table 4: Mean (±S.E) total plate count and coliform count in butter samples of different brands

		Total Plate Count			**Coliform count**			**Staph aureus count**		
	Samples	Means ± S.E	Min	Max	Means ± S.E	Min	Max	Means ± S.E	Min	Max
Gourmet	Butter	5.39 ±0.524	4.79	5.76	5.21±0.112	5.13	5.33	5.35 ±0.736	4.51	5.85
Haleeb	Butter	5.05 ±0.572	4.66	5.71	5.36±0.342	5.28	5.45	4.58 ±0.196	4.36	4.71
Nestle	Butter	3.67 ±0.349	3.41	4.07	4.79±0.327	4.52	5.17	3.60 ±0.167	3.41	3.71
gourmet,	ice cream	5.76 ±0.690	5.34	4.56	5.41±0.616	4.71	5.86	6.38 ±0.536	5.76	6.85
Walls	ice cream	4.31 ±0.275	4.11	4.63	3.79±0.370	3.51	4.21	3.90 ±0.480	3.49	4.43
Yummy	ice cream	4.33 ±0.654	3.62	4.91	4.05±0.710	3.34	4.76	4.85 ±0.595	4.27	5.46
Haleeb	milk powder	3.94 ±0.638	3.54	4.68	3.95±0.873	3.39	4.96	4.43 ±0.425	3.96	4.79
Nurpur	milk powder	4.87 ±0.630	4.25	5.51	4.16±0.826	3.21	4.71	4.89 ±0.299	4.65	5.23

Table 5: Chemical adulterants in yoghurt samples of different brands

Yoghurt Nurpur	**Urea**	**Formaldehyde**	**H_2O_2**	**Neutralizers**	**Starch**	**Boric Acid**	**QAC**
Yoghurt Haleeb	–ve	–ve	+ve	–ve	–ve	–ve	–ve
Yoghurt Nestle	–ve	–ve	+ve	–ve	–ve	–ve	–ve
Milk powder Nurpur	–ve	–ve	+ve	–ve	–ve	–ve	–ve
Milk powder Haleeb	–ve	–ve	+ve	–ve	–ve	–ve	–ve
Ice cream Gourmet	–ve	–ve	+ve	–ve	–ve	–ve	–ve
Ice cream Walls	–ve	–ve	+ve	–ve	–ve	–ve	–ve
Ice cream Yummy	-ve	–ve	+ve	–ve	–ve	–ve	–ve

4. Conclusion

On the basis of present findings, it is concluded that multiple factors can markedly affect the quality of dairy products. Adulteration of dairy products with chemicals such as Caustic soda, Urea, Antibiotics and Microbiological contamination such as Total plate count, Coliform count and *Staphylococcus aureus* in processed dairy products samples constitute a potential public health hazard.

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Declarations

The authors of the manuscript declare that they have no conflict of interest.

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