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Research Article

Water adulteration influences the physical characteristics of milk

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Abstract

In the Pakistan milk is marketed by traditional methods, whereby informal channels cause unethical activities of adulteration in the milk. In this regards, investigation was conducted. First phase included screening of market milk for various adulterants at three different zones (Southern, Central and Northern) of Sindh province, while in second phase *in vitro* study was conducted at the Department of Animal Products Technology, Sindh Agriculture University Tandojam to examine the effect of extraneous water on physical characteristics of milk. Results showed significant ($P<0.05$) effect of different concentrations of spiked water on physical characteristics of milk. In contrast to control milk (CM), physical characteristics of milk like specific gravity (sp. gr.), viscosity, conductivity, acidity, freezing point (FP) and refractive index (RI) of water spiked milk gradually decreased and pH values went towards neutral point with increase in concentration of water (10, 20, 30, 40 and/or 50%). Spiked source of water viz. water supply water (WSW) v/s canal water (CW), pond water (PW) and/or commercial ice water (CIW), and underground water (UW) v/s CIW showed considerable ($P<0.05$) variation in the sp. gr. of milk. Comparatively high ($P<0.05$) values were recorded for viscosity and acidity with spiked source of CW in milk, and for conductivity and RI with WSW versus their corresponding source of water. The pH values of milk admixed with PW and WSW recorded slightly alkaline, and with CW, UW and CIW slightly acidic. FP of milk added with WSW, CIW and/or with CW varied significantly ($P<0.05$) from each other.

Keywords: Adulteration; Extraneous water; Milk; Physical characteristics

Introduction

Adulteration is an act of internationally debasing the quality of food offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredients **[1]**. In Pakistan including Sindh province, raw milk is distributed by a traditional system through different intermediaries; whereby various adulterations in milk may take to maximize the profit. In this regards some literatures were reviewed and information related to adulteration was noted **[2]**.

One of the main adulterations found in milk is addition of extraneous water. This practice is usually used to increase the volume of milk. The literature regarding in this context indicates that dairy shop keepers add extraneous water to milk to earn more money from increased volume of milk [3]. This practice was mostly found in summer months for the purpose to increase the shelf life of milk, when temperature becomes very high. The dirty ice cubes have also been reported to be added in milk to lower down its temperature [4]. The extent of water adulteration up to 93.33% was reported in milk supplied to the various hospitals at Faisalabad city and it was reported 5.20, 8.80 and 18.80% to milk shops, hotels and cycle venders at Latifabad [5]. However, Awais [6] reported that all the milk samples (100%) collected from vicinity of Hyderabad city were adulterated with extraneous water. Similarly, 100% of extraneous water was reported in milk used for consumption at the vicinity of Badin city [7]. In another study, apparently similar percent (100) of water adulteration was noted in market milk at Hyderabad and its adjacent areas. However, the level of extraneous water in the market milk of Hyderabad remained 21.18%, while in its adjacent areas; it was reported as 17.75% [8]. Munir *et al.* [9] applied standard procedures in the determination of water ruining in milk sold at Peshawar, and its impact on chemical quality of milk. They divided the control group into four categories (i) pure milk with no added water; (ii) 80% milk and 20% added water; (iii) 60% milk and 40% added water; and (iv) 30% milk and 70% added water. Authors found that the average fat, solid not fat (SNF) and total solids (TS) contents of pure buffalo milk (7.53, 9.72 and 17.25%, respectively) were slightly different from samples with 20, 40 and 70% added water. The TS levels of milk with 20, 40 and 70% added water were 13.52, 10.13 and 5.64%, whilst SNF 7.66, 5.70 and 3.44% and fat percent 5.87, 4.43 and 2.20%, respectively. These results were used by the authors to estimate the extent of milk dilution of the market samples. They concluded that all the market samples did not reach the levels obtained for pure milk; they had all been either diluted or removed the constituents from it. While, in China, milk dealers adulterated the milk with water due to high demand and limited supply [10]. However, [11] reported the adulteration of milk with water at Kolkata state of India. They found 64.52% milk samples positive for extraneous water over 48 milk samples. Water adulteration in milk was also investigated at Sudan where by [12] examined 30 samples of market milk from retailers in Hilat kuku, Alwihda and Alsalama market and the control from the farm of Sudan University of Science and Technology. Author compared the specific gravity and the fat content of milk samples of Hilat kuku, Alwihda and Alsalama market (1.032, 1.033, and 1.032, respectively) and (4.1, 3.9, 4.3 and 3.5%, respectively) with control (1.032 and 3.5%, respectively) and found non-significant differences at all locations. There was no significant addition of water or skimming at all locations of Hilat kuku, Alwihda market, Alsalama [13]. In another study [14] inspected the status of adulteration and hygenesity of milk sold in state of Turkey. He reported that the majority of milk samples had very poor hygienic status, whereby 30% samples were adulterated with contaminated water.

Although various studies have already been conducted on milk adulteration with respect to different adulterants in Pakistan as well worldwide. Studies on the water adulteration is also well reported in the literature, however physical characteristics of milk with respect to water adulteration has rarely been investigated. Especially in the Sindh province of Pakistan such kind of studies have never been conducted. Current study was therefore planned, whereby extent of water adulteration in the different regions of Sindh province of Pakistan was be focused, further physical characteristics of milk with respect to extraneous water addition were studied

Materials and methods

Present investigation was conducted in two phases. In the first phase, market milk was screened for water adulteration at three different zones (Southern, Central and Northern) of Sindh province pf Pakistan. In the second phase, *in vitro* study was conducted at the Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University Tandojam to observe the effect of extraneous water on physical characteristics of milk.

Study area

Study area in the present investigation covered three different zones (Southern, Central and Northern) of Sindh province though were identified over milk marketing and consumption pattern, whereby three vicinities from each zone were selected. The selection of the vicinities within each zone is illustrated in (Fig. 1).

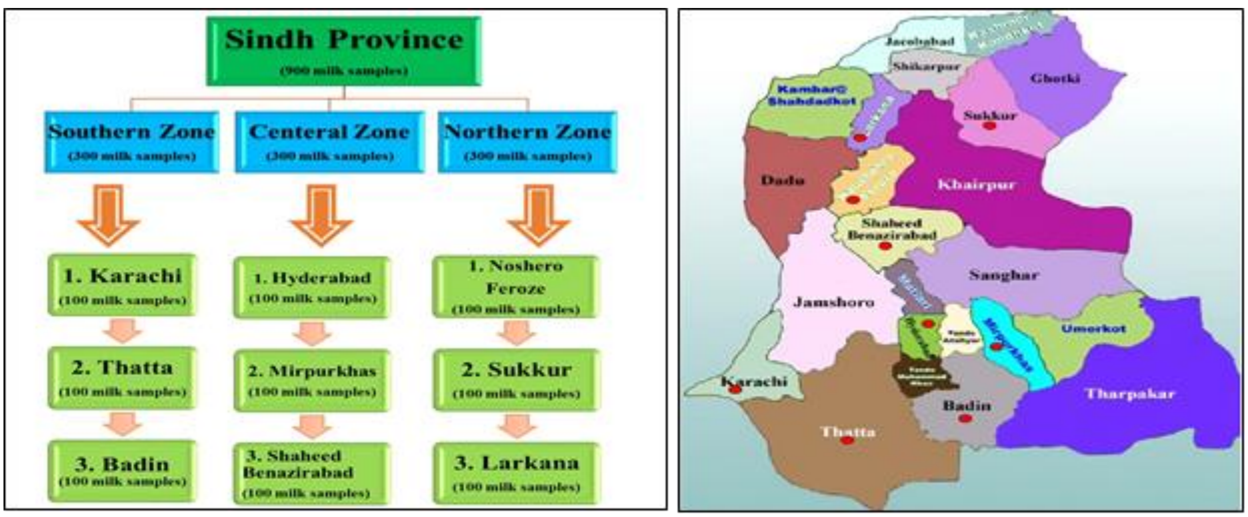

Figure 1. Flow chart showing the study area for milk sampling of milk for the analysis of various adulterants

Experimental procedure for screening of adulterants from market milk

Present investigation was conducted on adulteration in market milk from production through ultimate consumption at Sindh province during the year 2018. Milk samples were collected from milk marketing intermediaries at three districts each of from three zones of Sindh province. A total of nine hundred (n=900) market milk samples, three hundred (n=300) from each zone were sampled. Further, over three hundred (n=300) milk samples, hundred samples (n=100) from each vicinity and/or sixty (n=60) from each intermediaries were gathered. All the market milk samples were screened for adulteration at the Department of Animal Products Technology, Sindh Agriculture University Tandojam whereby adulterant like extraneous water was targeted.

Detection of extraneous water and its extent

Presence of extraneous water in market milk was observed according to the method of Association of Official Analytical Chemists **[15]**. The depression of freezing points of market milk and authentic milk samples (as freezing point base) was recorded using Cryoscope. The observed values of freezing point of market milk greater than that of authentic sample was assumed as presence of extraneous water in market milk. However, the extent of extraneous water in market milk was detected using following formula:

$$\text{Extraneous water \%} = \frac{\text{Freezing point base} - \text{observed freezing point}}{\text{Freezing point base}} \times 100$$

Analytical approach

The data so obtained was gathered and tabulated and the computerized statistical package *i.e.* Student Edition of Statistix (SXW), Version 8.1 (Copyright 2005, Analytical Software, USA) was approached. The data were initially processed through statistical procedure of summary statistics under which descriptive statistics was applied to observe the variability in data. The data was further analyzed through statistical procedure of analysis of variance (ANOVA) to observe the significant differences among the variables and in case of the significant differences appeared among the means, the least significant difference (LSD) test at 5% level of probability was applied [16].

Influence of water adulteration on physical characteristics of milk

A total of five water categories (*i.e.* Pond/tank water, underground water, canal water, water supply water and commercial ice water) with five concentration levels (*i.e.* 10, 20, 30, 40 and 50%) were spiked in whole milk, and their influence was observed against control (whole buffalo milk). The Whole milk samples were initially divided into 6 parts and grouped into A, B, C, D, E and F. Group A was kept as controlled, while rest of the five groups were again divided in to five (5) sub-groups each included B1, B2, B3, B4 and B5; C1, C2, C3, C4 and C5; D1, D2, D3, D4 and D5; E1, E2, E3, E4 and E5; F1, F2, F3, F4 and F5. Further, all of these five sub-groups were impaled with Pond/tank water, underground water, canal water, water supply water and commercial ice water each of with concentration of 10, 20, 30, 40 and 50%, respectively. The experiment was randomized with 6 × 5 factorial design and replicated with three times. All the samples were analyzed for physico-chemical, microbiological and sensorial characteristics. The obtained data were tabulated, and subjected to analysis of variance (ANOVA) and least significant difference (LSD) test to observe any significant difference among the variables (Gomez and Gomez, 1984).

Physical characteristics

Specific gravity

Specific gravity of spiked milk samples was determined according to the method of AOAC (2000) using pycnometer. The density of milk was measured against the density of standard (water). The pre-weighed pycnometer was filled with standard reference fluid (distilled water) to some pre-determined level at 20ºC and weight was taken. Similarly, pre-prepared milk sample was filled in pycnometer at similar level and temperature, and weighed. Specific gravity of milk was calculated by the following formula:

$$\textbf{Specific gravity} = \frac{\textbf{Weight of milk sample at 20°C}}{\textbf{Weight of distilled water at 20°C}}$$

Viscosity

Viscosity of spiked milk samples was determined according to the method of AOAC (2000) using viscometer with a uniform bore at 20°C. The flow of given volume of milk was compared with the rate of flow of the same quantity of water. Finely, the viscosity of water spiked milk sample was calculated according to the following formula:

$$\text{Viscosity (cP)} = \frac{\text{Flow time of milk at } 20^{\circ}\text{C} \times \text{Specific gravity of milk} \times 1.002}{\text{Flow time of water at } 20^{\circ}\text{C}}$$

Conductivity (mS/cm)

The conductivity of spiked milk samples was recorded using conductivity meter according to the method of AOAC (2000). Spiked milk sample was taken into a beaker and the probe of conductivity meter was inserted to the sample. The constant reading appeared on the screen of conductivity meter was noted, and recorded as conductivity value of milk sample.

Titratable acidity

The Titratable acidity of spiked milk samples was determined according to the method of AOAC (2000). Spiked milk sample (9ml) with phenolphthalein (3drops) was titrated using N/10 (0.01N) NaOH solution till appearance of light pink colour. The volume of N/10 NaOH solution used in the titration was noted. Thereafter following formula was applied to calculate the acidity percent in water spiked milk.

$$\text{Acidity \%} = \frac{\text{Volume of N/10 NaOH used} \times 0.009 \times 100}{\text{Volume of milk used}}$$

pH value

The pH value of water spiked milk samples was recorded according to the method of AOAC (2000) using pH meter. Spiked milk sample was taken into a beaker and electrode along with temperature probe was inserted to sample. The constant reading appeared on pH meter base was noted and recorded as pH value of milk sample.

Freezing point

Freezing point of water spiked milk samples was determined according to the method of (AOAC, 2000).

Refractive index

The refractive index of water spiked milk samples was recorded according to the method of AOAC (2000) using Refractometer. With the help of pipette 1 drop of water spiked milk sample was loaded on the sample area of refractometer. The constant reading appeared on the screen of refractometer was noted, and recorded as refractive index of milk sample.

Confirmation of spiked concentration of water in milk through specific gravity

Extent of spiked water in milk was computed from specific gravity of control milk versus specific gravity of water spiked milk. In this context, following formula was developed.

$$\text{Extent of spiked water} = \frac{\left[\text{Sp.gr. of control milk} - \text{Sp.gr. of standard } (H_2O)\right] - \left[\text{Sp.gr. of water adulterated milk} - \text{Sp.gr. of standard } (H_2O)\right]}{\left[\text{Specific gravity. of control milk} - \text{Sp.gr. of standard } (H_2O)\right]} \times 100$$

Confirmation of spiked concentration of water in milk through viscosity

Formula was developed on the basis of viscosity of control milk versus viscosity of water adulterated milk to calculate the spiked percent of extraneous water in adulterated milk. The formula is given bellow:

$$\text{Extent of spiked water} = \frac{\left[\begin{array}{c}\text{Viscosity control milk-}\\ \text{Viscosity of standard } (H_2O)\end{array}\right] - \left[\begin{array}{c}\text{Viscosity of water adulterated}\\ \text{milk-Viscosity of standard } (H_2O)\end{array}\right]}{\left[\text{Viscosity of control milk - Viscosity of standard } (H_2O)\right]} \times 100$$

Confirmation of spiked concentration of water in milk through conductivity

A formula on the basis of conductivity was innovated to calculate the spiked percent of water in adulterated milk. In this regard, the conductivity of control milk v/s conductivity of adulterated milk was computed. Following formula was applied:

$$\text{Extent of spiked water} = \frac{\left[\begin{array}{c}\text{Conductivity control milk-}\\ \text{Conductivity of standard } (H_2O)\end{array}\right] - \left[\begin{array}{c}\text{Conductivity of water adulterated}\\ \text{milk-Conductivity of standard } (H_2O)\end{array}\right]}{\left[\text{Conductivity of control milk - Conductivity of standard } (H_2O)\right]} \times 100$$

Confirmation of spiked concentration of water in milk through refractive index

Refractive index was used as tool for detection of spiked percent of extraneous water in adulterated milk. The refractive index of control milk was compared with refractive index of water adulterated milk. Formula used for this purpose is given bellow:

$$\text{Extent of spiked water} = \frac{\left[\begin{array}{c}\text{Ref. index of control milk-}\\ \text{Ref. index of standard } (H_2O)\end{array}\right] - \left[\begin{array}{c}\text{Ref. index of water spiked milk-}\\ \text{Ref. index of standard } (H_2O)\end{array}\right]}{\left[\text{Refractive index of control milk-Refractive index of standard } (H_2O)\right]} \times 100$$

Results and discussion

Influence of extraneous water on physical characteristics of milk

Results regarding the influence of source of water on physical characteristics of milk are shown in (Table 1). It was observed that specific gravity of milk with commercial ice water (1.0224), canal water (1.0221) and/or pond water (1.0221) recorded statistically similar (P>0.05) to one another. Further, the differences in specific gravity of milk spiked with underground water (1.018) v/s pond water (1.0221), underground water v/s canal water (1.0221), and underground water v/s water supply water (1.0215) existed non-significant (P>0.05), while, considerable difference occurred in the specific gravity of milk spiked with water supply water v/s canal water, pond water and/or commercial ice water, and underground water v/s commercial ice water. It was further noted that specific gravity of milk spiked with pond water, canal water and/or with commercial ice water was relatively similar to that of observed at mid concentration (*i.e.* 30%) of spiked water (1.022) as mentioned in (Table 1). Present findings are in agreement with that of **[17]**, who reported the lower specific gravity (1.0139) of high water adulterated milk obtained from milk vendors. In another study the specific gravity of milk from different agencies;

direct seller (1.026), milk collection center (1.026), milk vendor shops (1.026) and hotels (1.027) were remarkably ($P<0.001$) lower than that of dairy farm milk (1.031) **[18]**.

There was significant variation in viscosity of milk spiked with different sources. Milk spiked with canal water appeared considerably high ($P<0.05$) in viscosity (1.79cP) followed by milk spiked with pond water (1.74cP), commercial ice water (1.69cP), water supply water (1.64cP) and underground water (1.60cP). It was noteworthy that only spiked milk with commercial ice water had viscosity similar to that of milk spiked with mid concentration (*i.e.* 30%) of water, while milk spiked with canal water and/or with pond water had high, viscosity and with underground water and/or water supply water had low viscosity than that of appeared in milk spiked with mid concentration (*i.e.* 30%) of water (1.69cP) (Table 1). The results of **[19]** are in agreement with the findings of present study, who reported that the viscosity of control milk (1.82cP) was comparatively higher than that of milk samples (1.49cP) collected from different marketing channels.

Similarly, the results shown in (Table 1) regarding the conductivity of milk spiked with underground water (3.31mS/cm) v/s with canal water (2.29mS/cm) and/or with pond water (3.14mS/cm) v/s commercial ice water (3.16mS/cm) were relatively similar ($P>0.05$) to each other. While the conductivity of milk spiked with water supply water (3.37mS/cm) appeared significantly high ($P<0.05$) from milk spiked with other sources of water. Further it was noted that the increase in the conductivity of milk admixed with water supply water followed by pond water might be due to high conductivity in water supply water (5.39mS/cm) and pond water (4.31mS/cm), while low level of conductivity was found in milk added with underground water for the reason that its (1.21mS/cm) conductivity is very low **[20]**.

Results presented in (Table 1) further reveal that acidity of milk spiked with canal water (0.157%) was considerably high followed by milk spiked with underground water (0.140%), commercial ice water (0.116%), water supply water (0.087%) and pond water (0.073%). Moreover, the acidity in milk spiked with pond water and/or water supply water was less than and milk spiked with underground water, canal water and/or with commercial ice water was greater than that of milk spiked with mid concentration (*i.e.* 30%) of water (0.111) (Table 1). Similarly, **[21]** reported that the acidity of milk admixed samples collected from different milk selling points in Hyderabad city and its surroundings was recorded comparatively lower (0.14 and 0.15%) against control (0.19%).

Results regarding the pH values of milk mentioned in (Table 1) indicate that milk spiked with pond water and water supply water had relatively similar ($P>0.05$) pH (7.01 and 7.03, respectively) and found slightly alkaline. Though, milk spiked with canal water was more intense with H^+ (6.72) followed by milk spiked with underground water (6.79) and commercial ice water (6.90). However, pH values appeared in milk spiked with all of these water sources varied from that of revealed in milk spiked with mid concentration (*i.e.* 30%) of water (6.89). The findings of present study are varied from the results of **[22]** who reported variation in pH value was observed when water was added to normal milk. However, small quantity of water (10% water) did not show any significant influence on pH value of milk (6.68), while higher quantity (20% water) had remarkable effect ($P<0.05$) on it (6.72) compared to that of whole milk (6.64). Addition of 20% water showed remarkable ($P<0.05$) increases in pH value.

It was further noted from (Table 1) that there were no significant differences in freezing point of milk spiked with pond water (-0.382°C) and underground water (-0.383°C), while freezing point of milk spiked with water supply water (-0.388°C), commercial ice water (-0.393°C) and/or with canal water (-0.408°C) significantly ($P<0.05$) varied from one another. Further, freezing point of milk spiked with pond water, underground water and canal water varied from that of shown in (Table 1) (-0.390°C) for milk spiked with mid concentration of water (*i.e.* 30%), and with that of water supply water and commercial ice water found very close to spiked concentration of water in milk. The results of **[23]** are in line with the findings of present investigation, who reported significant impact of extraneous water on the freezing point of normal whole milk (-0.525˚C), when 10% or 20% water (-0.462 and -0.399˚C, respectively) was added to it.

Refractive index of milk spiked with pond water (1.3436), underground water (1.3436) and/or canal water (1.3436) was apparently similar and found significantly ($P<0.05$) lower from that of milk spiked with water supply water (1.3440) and higher from that of spiked with commercial ice water (1.3431). The findings of **[24]** are in line with the results of present study, they reported that the refractive index of milk is directly related with its total solid content.

Table 1. Influence of source of extraneous water on physical characteristics of milk

Spiked source of water in milk	Physical characteristics						
	Specific gravity	**Viscosity (cP)**	**Conductivity (mS/cm)**	**Acidity (%)**	**pH value**	**Freezing point (˚C)**	**Refractive index**
Pond water	1.0221^{ab}	1.74^{b}	3.14^{c}	0.073^{e}	7.01^{a}	-0.382^{a}	1.3436^{b}
Underground water	1.0218^{bc}	1.60^{e}	3.31^{b}	0.140^{b}	6.79^{c}	-0.383^{a}	1.3436^{b}
Canal water	1.0221^{ab}	1.79^{a}	2.29^{b}	0.157^{a}	6.72^{d}	-0.408^{d}	1.3436^{b}
Water supply water	1.0215^{c}	1.64^{d}	3.37^{a}	0.087^{d}	7.03^{a}	-0.388^{b}	1.3440^{a}
Commercial ice water	1.0224^{a}	1.69^{c}	3.16^{c}	0.116^{c}	6.90^{b}	-0.393^{c}	1.3431^{c}
LSD (0.05)	0.5109	0.0104	0.0262	0. 3619	0.0365	0.2566	0.0034
SE±	0.2580	0.0524	0.0132	0.1828	0.0185	0.1295	0.0017

Means with different letters in same column varied significantly from one another; Data of each adulterant are the average of five concentrations

Influence of different concentrations of extraneous water on physical characteristics of milk

It was noted from (Table 2) that different concentrations of water had significant influence on physical characteristics of milk. The specific gravity, viscosity, conductivity, freezing point and refractive index of control milk appeared as 1.032, 2.23cP, 4.38mS/cm, -0.547°C and 1.3473, respectively. However, when water was spiked in milk with concentration of 10, 20, 30, 40 and 50%, there was gradual and more or less linear decrease in these variables. For instance, in contrast to whole milk (1.032), the decrease in water spiked milk (10, 20, 30, 40 and 50%) was recorded as 1.028, 1.025, 1.022, 1.019 and 1.016, respectively for specific gravity, 1.94, 1.80, 1.69, 1.57 and 1.46cP, respectively for viscosity, 3.89, 3.57, 3.25, 2.93 and 2.61mS/cm, respectively for conductivity,

-0.501, -0.445, -0.390, -0.336 and -0.282°C, respectively for freezing point and 1.3450, 1.3443, 1.3429, 1.3415 and 1.3400, respectively for refractive index. Similarly, **[25]** found significant variation in the specific gravity of whole milk (control) (1.030) and milk added with 10% water (1.027) and 20% water (1.024). The findings of **[26]** are in accordance with results of present investigation, who found lower values of viscosity in milk samples (1.26±0.01and 1.46±0.01) collected from milk collecting units at Hyderabad and its vicinities. Further, results showed that the acidity of control milk was 0.126% and it was relatively similar to that of water spiked milk with 10% (0.124%), while acidity in both of these control and spiked milk with 10% water were comparatively ($P<0.05$) varied from that of milk spiked with 20, 30, 40 and 50% water (0.114, 0.111, 0.112 and 0.113%, respectively) though appeared statistically non-significant ($P>0.05$) to each other in acidity. While in agreement with the results of present study **[27]** reported low level (0.05 to 0.07%) acidity in milk supplied by milk dealers to public places and Tea stalls at the Faisalabad city.

pH value of spiked milk with 10% water (6.85) was noted statistically similar ($P>0.05$) to that of milk spiked with 20% water (6.88), but it varied significantly ($P<0.05$) in pH values from that of milk spiked with 30, 40 and/or 50% water (6.89, 6.89 and 6.93, respectively). Nevertheless, the pH values all of these spiked milk were significantly different ($P<0.05$) from that of control milk (6.78). Similarly, in another study **[28]** found significant variation among the pH values (6.54, 6.53, 6.65 and 6.66) of milk samples collected from different milk traders in the surrounding of Tandojam city.

Table 2. Influence of different extraneous water concentrations on physical characteristics of milk

Spiked concentration (%) of water in milk	Physical characteristics						
	Specific gravity	Viscosity (cP)	Conductivity (mS/cm)	Acidity (%)	pH value	Freezing point (˚C)	Refractive index
Control	1.032^{a}	2.23^{a}	4.38^{a}	0.126^{a}	6.78^{d}	-0.547^{f}	1.3473^{a}
10	1.028^{b}	1.94^{b}	3.89^{b}	0.124^{a}	6.85^{c}	-0.501^{e}	1.3457^{b}
20	1.025^{c}	1.80^{c}	3.57^{c}	0.114^{b}	6.88^{bc}	-0.445^{d}	1.3443^{c}
30	1.022^{d}	1.69^{d}	3.25^{d}	0.111^{b}	6.89^{ab}	-0.390^{c}	1.3429^{d}
40	1.019^{e}	1.57^{e}	2.93^{e}	0.112^{b}	6.89^{ab}	-0.336^{b}	1.3415^{e}
50	1.016^{f}	1.46^{f}	2.61^{f}	0.113^{b}	6.93^{a}	-0.282^{a}	1.3400^{f}
LSD (0.05)	0.0048	0.0152	0.0361	0.5258	0.0348	0.0027	0.0038
SE±	0.0024	0.7692	0.0183	0.2661	0.0176	0.0013	0.0019

Means with different letters in same column varied significantly from one another; Data in each concentration are the average of six replications of five adulterants

Interactive influence of different sources v/s concentrations of water on the physical characteristics of milk

In (Table 3) shows the interactive influence of source v/s concentration of water on physical characteristics of milk. Result reveals that in contrast to control milk there were significant ($P<0.05$) interactive influence of spiked source v/s concentration of water on specific gravity, viscosity, conductivity, freezing point and refractive index of milk. However, there were no significant ($P>0.05$) interactive influence of spiked source of water *i.e.* pond water, underground water, canal water, water supply water and commercial ice water each of @ 10, 20, 30, 40 and 50% on specific gravity of milk. While significant effect existed on specific gravity with increase in concentration (from 10 up to 50%) of spiked water in each source. Results further indicate that there were non-significant interactive influence (P>0.05) of source of water v/s concentration on viscosity of milk spiked at 10% water supply water (1.96cP) v/s pond water (1.97cP), and both of these were significantly (P<0.05) varied from that of milk spiked with underground water (1.83cP), spiked commercial ice water (1.92cP) and/or with canal water (2.02cP) at similar concentration of water *i.e.*10%. Similar trend of interactive influence on viscosity of milk existed at spiked concentration of 20, 30, 40 and 50% water v/s each source of water. The reason behind the low level of viscosity in market milk samples is eventually due to addition of extraneous water or subtraction of valuable fat/cream from milk **[29]**. The findings of **[30]** researcher found low specific gravity in milk samples collected from Bhangnamari bazaar (1.026), Sutiakhli bazaar (1.027), Vabokhali bazaar (1.027) and Mymensingh sadar bazaar (1.024) milk were lower than that of control 1.032 and the decrease in the specific gravity may be the addition of extraneous water in market milk by milk traders. Moreover, in the present study the variation was observed in the specific gravity of milk admixed with different water categories, however, it was found to be higher in the milk admixed with canal water, because the turbidity of canal water is high due to suspended muddy material, solids leads, heavy metals, total soluble salts and domestic waste water discharged in it from environment **[31]**.

In (Table 3) further shows that spiked source of water at 10% level in milk like pond water, canal water, water supply water and commercial ice water (0.127, 0.125, 0.117 and 0.125%, respectively) had no significant effect (P>0.05) on acidity of milk, though were relatively similar to that of control milk (0.127%), while acidity of milk spiked with underground water (0.130%) at similar concentration (10% level) appeared significantly (P<0.05) different from them. However, interactive influence of source v/s concentration of water in milk at 20 and 30% level varied from above said concentration (*i.e.* 10% level), whereby acidity of milk spiked with canal water and water supply water (0.125 and 0.133%, respectively) at 20% level and with pond water and canal water (0.125 and 0.138%, respectively) were relatively similar (P>0.05) to that of control milk, while in other source of water in both cases it was significantly (P<0.05) different from that of control milk. It was further noted that interactive influence of source v/s concentration of water on acidity of milk was comparatively (P<0.05) different at 40 and 50% level, whereby acidity of milk due to all spiked sources of water in milk (*i.e.* pond water, underground water, canal water, water supply water and/or commercial ice water) (*i.e.* 0.108, 0.06, 0.147, 0.180, 0.070, and 0.105, 0.048, 0.167, 0.205 and 0.05%, respectively) were significantly (P<0.05) different from that of control milk. Results presented in (Table 3) reveal that in contrast to control milk, there were non-significant variation (P>0.05) in pH values of milk spiked with either pond water, canal water and/or water supply water at 10% level (6.78, 6.78 and 6.83, respectively) and at 20% level (6.85, 6.80 and 6.78, respectively), with canal water at 30, 40 and 50% (6.79, 6.78 and 6.79, respectively) and with water supply water at 30% level (6.73%). However, the pH values of milk spiked with each concentration level other than above said sources of water found significantly (P<0.05) different from that of control milk. Present findings are also in accordance with the results of **[32]** who reported that the average freezing point of market milk samples of Hyderabad and its surrounding areas was evaluated as -0.428 and - 0.447% against control (-0.543°C).

Table 3. Interactive influence of source v/s concentrations of water on the physical characteristics of milk

Spiked water in milk		Physical characteristics						
Source	**Concentration (%)**	**Specific gravity**	**Viscosity (cP)**	**Conductivity (mS/cm)**	**Acidity (%)**	**pH values**	**Freezing point (°C)**	**Refractive index**
Control	**0**	1.0317^{a}	2.23^{a}	4.39^{a}	0.127^{efg}	6.78^{ij}	-0.547^{p}	1.3473^{a}
Pond water	10	1.0282^{b}	1.97^{c}	3.78^{cd}	0.127^{efg}	6.78^{ij}	-0.493^{m}	1.3457^{bc}
	20	1.0252^{c}	1.86^{e}	3.46^{f}	0.150^{d}	6.85^{ghi}	-0.438^{j}	1.3443^{de}
	30	1.0220^{d}	1.75^{h}	3.14^{hi}	0.125^{fg}	6.89^{fgh}	-0.380^{g}	1.3429^{fg}
	40	1.0190^{e}	1.63^{k}	2.82^{k}	0.108^{hi}	6.90^{fgh}	-0.325^{d}	1.3415^{hij}
	50	1.0160^{f}	1.52^{m}	2.50^{m}	0.105^{hij}	6.93^{ef}	-0.272^{a}	1.3401^{lm}
Underground water	10	1.0280^{b}	1.83^{ef}	3.95^{b}	0.103^{ij}	6.93^{ef}	-0.495^{mn}	1.3458^{bc}
	20	1.0248^{c}	1. 72^{hi}	3.63^{e}	0.083^{kl}	6.98^{cde}	-0.438^{j}	1.3443^{de}
	30	1.0217^{d}	1.61^{k}	3.31^{g}	0.073^{lm}	7.03^{bcd}	-0.382^{g}	1.3429^{fg}
	40	1.0188^{e}	1.49^{m}	2.99^{j}	0.060^{no}	7.05^{bc}	-0.330^{d}	1.3415^{hij}
	50	1.0158^{f}	1.38^{o}	2.67^{l}	0.048^{o}	7.08^{b}	-0.273^{a}	1.3401^{lm}
Canal water	10	1.0282^{b}	2.02^{b}	3.92^{b}	0.125^{fg}	6.78^{ij}	-0.520^{o}	1.3457^{bc}
	20	1.0252^{c}	1.91^{d}	3.61^{e}	0.125^{fg}	6.80^{ij}	-0.461^{l}	1.3443^{de}
	30	1.0222^{d}	1.79^{g}	3.29^{g}	0.138^{de}	6.79^{ij}	-0.408^{i}	1.3429^{fg}
	40	1.0190^{e}	1.68^{j}	2.97^{j}	0.147^{d}	6.78^{ij}	-0.353^{f}	1.3415^{hij}
	50	1.0162^{f}	1.57^{l}	2.65^{l}	0.167^{c}	6.79^{ij}	-0.300^{c}	1.3401^{lm}
Water supply water	10	1.0275^{b}	1.96^{c}	4.00^{b}	0.117^{gh}	6.83^{hi}	-0.500^{n}	1.3462^{bc}
	20	1.0247^{c}	1.73^{h}	3.69^{de}	0.133^{ef}	6.78^{ij}	-0.440^{j}	1.3448^{d}
	30	1.0215^{d}	1.62^{k}	3.36^{g}	0.150^{d}	6.73^{jk}	-0.390^{h}	1.3434^{f}
	40	1.0185^{e}	1.50^{m}	3.05^{ij}	0.180^{b}	6.67^{kl}	-0.330^{d}	1.3420^{hi}
	50	1.0155^{f}	1.39^{o}	2.72^{l}	0.205^{a}	6.60^{l}	-0.280^{b}	1.3406^{kl}
Commercial ice water	10	1.0285^{b}	1.92^{d}	3.80^{c}	0.125^{fg}	6.91^{efg}	-0.500^{n}	1.3452^{cd}
	20	1.0255^{c}	1.81^{fg}	3.48^{f}	0.103^{ij}	6.96^{def}	-0.448^{k}	1.3437^{ef}
	30	1.0223^{d}	1.69^{ij}	3.16^{h}	0.087^{k}	7.02^{bcd}	-0.391^{h}	1.3423^{gh}
	40	1.0193^{e}	1.58^{l}	2.84^{k}	0.070^{mn}	7.04^{bc}	-0.340^{e}	1.3409^{j}
	50	1.0163^{f}	1.46^{n}	2.52^{m}	0.050^{o}	7.21^{a}	-0.285^{b}	1.3305^{m}
LSD (0.05)		0.0109	0.0340	0.0885	0.0118	0.077	0.00608	0.0245
SE±		0.0552	0.0172	0.0448	0.0595	0.039	0.00308	0.0124

Means with different letters in same column varied significantly from one another

Results regarding freezing point as well as refractive index of milk mentioned in (Table 3) indicate that addition of water in milk with each of five sources of water (pond water, underground water, canal water, water supply water and commercial ice water) @ 10, 20, 30, 40 and 50% level gave significantly ($P<0.05$) comparable levels against the control. Interactive influence of source of water v/s concentration found prominent on most of the cases of freezing point of milk, while it was rarely appeared on refractive index of milk. Freezing point of milk spiked with pond water (-0.493°C) v/s underground water (-0.495°C) and/or with water supply water (-0.500°C) v/s commercial ice water (-0.500°C) noted relatively similar (P>0.05) to each other at concentration level of 10% (-0.493°C v/s -0.495°C and/or -0.500°C v/s -0.500°C, respectively), 30% (-0.380°C v/s -0.382°C and/or -0.325°C v/s -0.330°C, respectively) and 50% (-0.272°C v/s -0.273°C and/or -0.280°C v/s -0.285°C, respectively), while both of above said two groups are significantly (P<0.05) different from one another, as well as from that of milk spiked with canal water (-0.520, -0.408 and -0.300°C, respectively). However, in case of milk spiked at 20 and 40% concentration level with pond water (-0.438 and -0.325°C, respectively) v/s underground water (-0.438 and -0.330°C, respectively) v/s water supply water (-0.440 and -0.330°C, respectively), the freezing point was statistically similar (P>0.05), and appeared significantly (P<0.05) different from that of milk spiked with canal water (-0.461 and -0.353°C, respectively) and/or with commercial ice water (-0.448 and -0.340°C, respectively). Results mentioned in (Table 3) indicate that there were non-significant (P>0.05) variation in refractive index of milk spiked with pond water, underground water, canal water, water supply water and/or commercial ice water at concentration level of 10 and 40%. While at concentration level of 20, 30 and/or 50%, the refractive index of milk spiked with water supply water (1.3448, 1.3434 1.3305, respectively) found significantly (P<0.05) different from that of milk spiked with commercial ice water (1.3437, 1.3434 and 1.3401, respectively) and relatively similar to that of spiked with pond water (1.3443, 1.3429 and 1.3401, respectively), underground water (1.3443, 1.3429 and 1.3401, respectively) and canal water (1.3443, 1.3429 and 1.3401, respectively). Similarly, [33] reported that the milk marketing agencies diluted the milk with extraneous water to gain more profit from increased volume of milk, while the physical quality like refractive index and density of milk is adversely decreased by this malpractice.

Extent in decrease in physical characteristics of milk spiked with different sources of water

The results related with extent in decrease in physical characteristics of milk spiked with different sources of water are illustrated in (Table 4). It was observed that decrease percent of specific gravity, viscosity, conductivity and refractive index of milk spiked with pond water, water supply water, canal water and/or commercial ice water was greater than expected percent of spiked water (The average five of concentration levels, *i.e.* 30%) except refractive index of milk spiked with water supply water, where decrease percent was less than that of spiked percent of water (*i.e.* 30%). Decrease percent in specific gravity of milk spiked with underground water (33.44%), although appeared slightly high contrast to that of spiked with pond water (32.68%) and water supply water (32.46%), and lower than that of canal water (34.43%), the differences among them existed non-significant (P>0.05), while it was significantly higher than that of milk spiked with commercial ice water (31.58%). In case of viscosity of milk, the decrease percent was significantly (P<0.05) higher in spiked underground water (54.43%) followed by with canal water (51.63%), commercial ice water (47.20%), pond water (42.37%) and water supply water (37.96%). The decline percent in conductivity of milk added with pond water (38.84%) v/s commercial ice water (38.22%) and/or with underground water (33.47%) v/s water supply water (34.19%)

each of found statistically similar (P>0.05). In accordance with the present investigation [34] reported that the addition of extraneous water and filthy ice by various milk intermediaries like milk collectors, middlemen, processors and retailer keepers may seriously affect the physical quality of milk by subtracting the valuable nutrients and adding with different proportions of inferior quality ingredients, such kind of unethical act may pose injurious effects on the end user/consumer health.

However, the decrease percent in conductivity of milk spiked with former two sources of water appeared considerably (P<0.05) high from that of later two sources of spiked water as well as from that of milk spiked with canal water (31.73%). There were no any significant differences (P>0.05) in the decrease in freezing point of milk spiked with pond water (30.26%) v/s underground water (30.10%) v/s canal water (29.25%). This decline percent in freezing point above said three spiked milks found relatively close to that of average percent of five concentration levels of spiked water in milk (*i.e.* 30%). Nevertheless, the freezing point was affected more with addition of water supply water whereby the decrease percent (25.30%) was remarkably (P<0.05) lower than that of above three spiked sources of water in milk as well as from that milk spiked with commercial ice water (28.33%). Similarly, the reduction percent in refractive index of milk added with pond water (31.27%) v/s underground water (31.08%) v/s water supply water (31.18%) was relatively similar (P>0.05) and appeared significantly (P<0.05) high from that of milk spiked with water supply water (27.69%) and considerably (P<0.05) less from that of milk added with commercial ice water (35.30%). Reported results of [32-35] are also in support to our findings.

Table 4. Extent in decrease in physical characteristics against spiked source of water in milk

Spiked source of water in milk (%)	Observed percent of physical characteristics				
	Specific gravity	Viscosity (cP)	Conductivity (mS/cm)	Freezing point (°C)	Refractive index
Pond water	32.68^{hi}	42.37^{d}	38.84^{e}	30.26^{klm}	31.27^{j-l}
Underground water	33.44^{gh}	54.43^{a}	33.47^{gh}	30.099^{lm}	31.08^{j-l}
Canal water	32.46^{h-j}	37.95^{e}	34.19^{fg}	25.30^{p}	31.18^{j-l}
Water supply water	34.43^{fg}	51.63^{b}	31.73^{ij}	29.25^{mn}	27.689^{o}
Commercial ice water	31.58^{i-k}	47.20^{c}	38.22^{e}	28.33^{no}	35.30^{f}

LSD (0.05) = 1.3965; **SE±** = 0.7111
Means with different letters in same row and column varied significantly from one another

Extent in decrease in physical characteristics of milk spiked with different concentration of water

Table 5 indicates the results regarding extent in decrease in physical characteristics of milk spiked with different concentrations of water. It was noted that there were significant (P<0.05) variation in decrease in each of specific gravity, viscosity, conductivity, freezing point and refractive index of milk spiked with similar as well as different concentration of water. This decrease in all above said characteristics of milk found greater than their corresponding spiked concentration of water except freezing point of water, whereby decrease existed the less. For instance, the extent in decrease against each spiked concentration of water (*i.e.* 10, 20, 30, 40 and 50%) appeared high in viscosity (25.28, 37.14, 47.06, 57.06 and 67.04%, respectively), followed by conductivity (15.37, 25.26, 35.32, 45.22 and 55.27%, respectively), specific gravity (12.94, 22.81, 33.11, 42.98 and 52.74%, respectively) and refractive index (11.43, 21.34, 31.25, 41.21 and 51.30, respectively) and low in freezing point (8.63, 18.67, 28.63, 38.71 and 48.60%, respectively). However, [30, 31, 34-37] also reported similar types of results.

Table 5. Extent in decrease in physical characteristics against their corresponding percent of spiked water in milk

Spiked concentration of water in milk (%)	Observed percent of physical characteristics				
	Specific gravity	Viscosity (cP)	Conductivity (mS/cm)	Freezing point (°C)	Refractive index
10	12.941^{v}	25.278^{q}	15.369^{u}	8.631^{x}	11.431^{w}
20	22.811^{r}	37.136^{l}	25.263^{q}	18.667^{t}	21.338^{s}
30	33.112^{n}	47.057^{g}	35.320^{m}	28.632^{p}	31.249^{o}
40	42.979^{i}	57.064^{b}	45.215^{h}	38.711^{k}	41.205^{j}
50	54.740^{d}	67.043^{a}	55.273^{c}	48.596^{f}	51.299^{e}

LSD (0.05) = 1.3965; **SE±=** 0.7111

Means with different letters in same row and column varied significantly from one another

Conclusion

Study concludes that water adulteration considerably impairs the physical quality of milk. Physical characteristics of milk such as specific gravity, viscosity, conductivity, acidity, freezing point and refractive index (RI) of water adulterated milk remain low, while pH values moves towards neutral point with increased water adulteration in milk.

Authors' contributions

Conceived and designed the experiments: GS Barham & AA Khaskheli, Performed the experiments: GS Barham, Analyzed the data: GB Khaskheli & AS Magsi, Contributed reagents/ materials/ analysis tools: AH Shah, Wrote the paper: AA Khaskheli.

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