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Extent of Extraneous Water and Detection of Various Adulterants in Raw Milk during Supply Chain and its Impacts on Milk Physical Characteristics

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ABSTRACT

Background: The study's objective was to recognize the various adulterants and their effects on the physical characteristics of the raw milk available in the city. The milk dealers, especially in the developing countries usually involved in milk adulteration practices in order to increase their profit margins, which poses significant health hazards.

Methods: A total of 189 samples of raw milk were gathered across Karachi's five districts. Milk adulteration testing kit were employed for various adulterants detection and its physical impacts on milk was analyzed by the AOAC methods.

Result: The milk samples from selling points had the highest levels of adulteration, followed by those from transportation cans and storage tanks at dairy farms. Chemical adulteration impact on milk pH and specific gravity was very prominent while extraneous water showed significant impact on milk specific gravity rather than its pH. Average pH and specific gravity values of milk sample collected from storage tanks at dairy farm were normal, while it was out of range in the milk samples collected from transportation cans and selling points. As indicated that at the dairy farms had very few milk adulteration activities, whereas milk samples being transported and sold in stores had the highest number of fraud attempts.

Key words: Adulteration, pH, Raw milk, Specific gravity, Supply chain.

Abbreviations: AOAC: Association of Official Agricultural Chemists; FSSAI: Food Safety and Standards Authority of India; UVAS: University of Veterinary and Animal Sciences Lahore, Pakistan.

INTRODUCTION

Adulteration in the raw milk is the main dilemma in Pakistan. In order to increase their profit margins, certain middlemen and retailers may employ inexpensive, adulterated or chemically infused substances, which poses significant health hazards (Javed, 2016). To make the milk denser and to increase its shelf life, the milkman also adds starch, flour, urea, vegetable oil and other ingredients (Tanveer, 2015). The adulteration not only affects the milk quality but may also pose serious threats to human health safety; *e.g.* ammonia in milk has been linked to immune system deterioration, renal issues and sensory difficulties. For instance, carbonate (HCO_3-) and bicarbonate can cause gastrointestinal issues, high blood pressure, renal failure, edema and cardiac failure, hydrogen peroxide (H_2O_2) disrupts the body's antioxidant defenses, natural immunity, acid-base balance and blood pH. while, formalin (CH_2O) is a strong carcinogen it causes vomiting, diarrhea and abdominal pain.

Benzoic acid ($C_7H_6O_2$) creates adverse effects such as asthma, urticaria and metabolic acidosis. Boric acid (H_3BO_3) causes nausea, vomiting, diarrhea, damage kidney, severe failure of circulatory system and even death, melamine ($C_3H_6N_6$) causes the urinary tract problems in infants and children (Barham *et al.*, 2014; Khan *et al.*, 2017).

During storage and shipment, the middleman adds ice blocks of contaminated water to prevent milk from spoilage (Ullah *et al.*, 2016).

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In undeveloped countries, open raw milk has been linked to numerous zoonotic illnesses. The most common public health issues globally are those related to foodborne illnesses. An estimated 600 million cases and 420,000 deaths worldwide are attributed to foodborne diseases

(Ayele *et al*., 2017). Although food scams have an economic inspiration, but their effects on public health are a serious issue (Banik *et al*., 2014). The situation is far worse abroad since there is inadequate law enforcement and monitoring (Azad and Ahmed, 2016). Thus, managing and maintaining milk quality is one of the primary difficulties facing the dairy business. In undeveloped countries, informal methods are used to distribute most raw milk sales (Aamir, 2018). With the idea of creating risk tracking techniques and their mitigation plans in the future, the study's goal was to identify the different adulterants and their impacts on the physical nature of raw milk available in the local market.

MATERIALS AND METHODS

Collection of samples

Milk samples were collected from numerous dairy farm's storage tanks; the same milk was recollected from the transportation cans and retail shop's selling point during its journey. Total 189 samples were collected throughout the city during the summer 2021 to study their quality. In accordance with the plan, 69 samples were gathered from a total of 21 retail shop's selling point, 90 samples were taken from various transportation cans and 30 samples were obtained from 10 dairy farm's storage tanks. District-wise, 36 samples were gathered from the Central district, compared to 39 samples from each of the East, West, North and South districts.

All the milk samples were collected in clean, dry, sterile polythene plastic bags and rushed immediately to the PCSIR laboratory Complex, Karachi under aseptic conditions.

Detection of various adulterants in milk samples

The detection of various adulterants in raw milk were carried out in accordance with the FSSAI:2015 lab 1 manual and the Standard Operation Procedure for milk adulteration testing kit (UVAS, 2020).

Extent of extraneous water in milk

The AOAC claims that the Cryoscope's depression of the freezing point can be used to detect the presence of extra water in milk. When milk's freezing point was higher than that of the control, it was considered that there was excess water present in the milk.

The amount of extraneous water in market milk was determined by measuring the depression of the freezing point (using a Cryoscope) and then used the following formula:

% of added water =

$$\frac{\text{Freezing point base - Observed freezing point}}{\text{Freezing point base}} \times 100$$

Physical analysis

pH value

A calibrated pH meter was used to determine the pH of market milk samples in accordance with (AOAC 2000). A beaker containing a sample of milk had electrodes and a temperature probe inside it. The pH of the milk sample was measured and logged along with the dependable reading that appeared on the pH meter's base.

Freezing point

The (AOAC, 2000) method was used to assess the freezing point of market milk samples. The Cryoscope was filled with a milk sample. The freezing point of the milk sample was determined by noting and recording the consistent reading that displayed on the 4C3 multi-samples Cryoscope's screen.

Specific gravity

Using a pycnometer, the specific gravity of samples of market milk was calculated in accordance with the (AOAC 2000) procedure. Milk's density was compared to a typical reference (water). The standard reference (water) was poured into the pre-weighed pycnometer to a predetermined level at 20°C and weight was recorded. In a similar manner, a pre-prepared milk sample was weighed after being filled in a pyrometer at a similar level and temperature. The specific gravity of milk was calculated using the formula shown below:

$$\text{Specific gravity} = \frac{\text{Weight of milk sample}}{\text{Weight of distilled water}}$$

Statistical analysis

The IBM SPSS Statistics version 22 software was used to analyze the data. In order to find differences between the variables that were statistically significant, the data was further evaluated using the statistical method of analysis of variance (ANOVA). In those cases, where such differences were discovered, the means were further computed using the least significant difference (LSD) at the 5% level of probability.

ANOVA

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.008497	2	0.004249	26.91869	0.012126	9.552094
Within groups	0.000474	3	0.000158			
Total	0.008971	5				

RESULTS AND DISCUSSION

Ratio of adulterated milk samples in each district

Complete milk supply chains in Karachi's five districts were examined for the presence of several adulterants, including cane sugar, starch, urea, ammonium, hydrogen peroxide, hypochlorite, sodium chlorite, carbonate, formalin, boric acid, detergent/soap and exogenous water. After 189 samples were analyzed, 76 (40%) samples were found positive, with 17 (22%) positive samples were found in each of the districts of East, West and North, 13 (17%) in South and 12 (16%) in Central.

Percentage of positive milk samples

When these samples were examined, the most common adulterants; urea, detergents, sodium chloride, starch, carbonate, hydrogen peroxide and formalin were found in the milk samples available in the market. Urea was found in 34% of the adulterated samples, detergents in 31%, cane sugar and starch in 9%, sodium chloride in 8%, carbonate in 5%, hypochlorite in 3% and formalin in 1% samples. The following adulterants like ammonium, hydrogen peroxide (H_2O_2) and boric acid (H_3BO_3) were not detected in any collected milk samples (Fig 1).

Percentage of extent of extraneous water in milk samples

Through the depression of freezing point, the amount of extraneous water was identified in milk samples obtained from various locations along the supply chain in each district. The average extent of added water in milk samples collected from dairy farm's storage tanks in district East was recorded as 10.23±2.17%, followed by samples collected from transportation cans (16.3±3.45%) and 24.60±2.47% in selling point milk samples. The average extent of added water in milk samples collected from dairy farm's storage tanks in district West was noted as 14.23±3.5%, 22.45±4.43% in transportation cans while 32.50±2.72% was noted in selling points milk samples.

Similarly, average 13.32±2.56 added water was detected in the milk samples collected from the storage tanks in district North, 21.74±4.65% was recorded in the transportation cans samples and 32.37±4.24% in the selling points milk samples. The average extent of added water in milk samples collected from dairy farm's storage tanks in district South was recorded as 18.36±4.47%, followed by transportation cans (32.6+3.28%) and 44.52±4.14% in

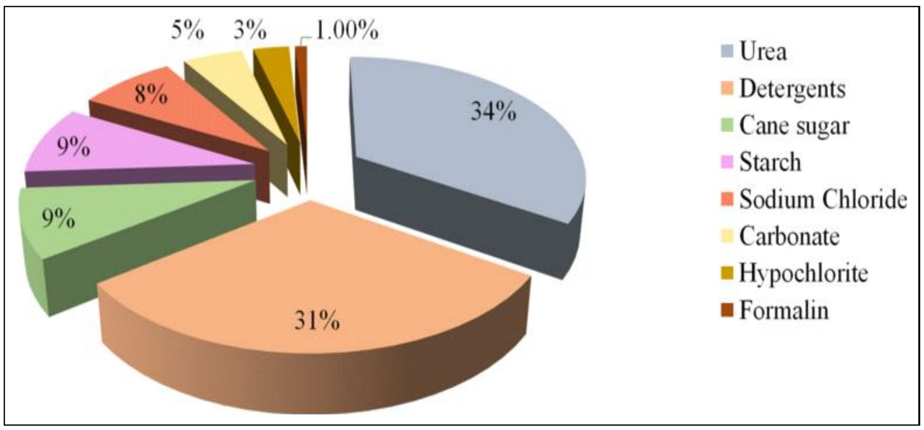
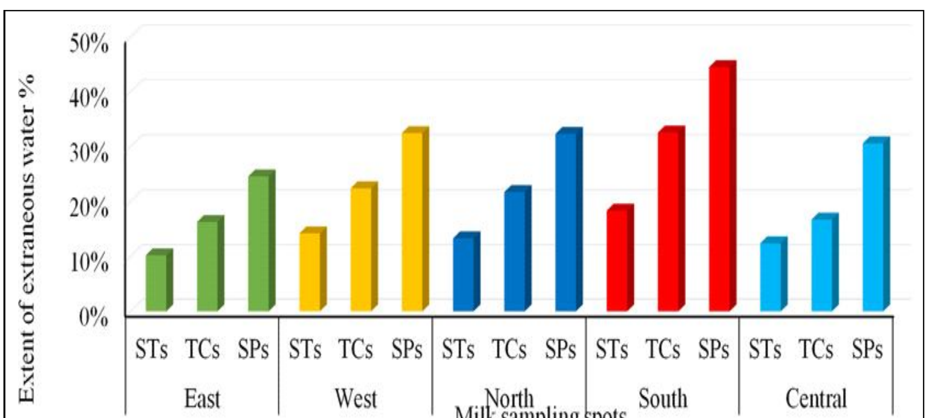


Fig 1: Percentage of adulterated milk samples with the common adulterants.



STs: Storage tanks, TCs: Transportation cans, SPs: Selling points.

Fig 2: Percentage of extraneous water in milk samples.

selling points samples. The average extent of added water in milk samples collected from dairy farm's storage tanks in district Central was recorded as 12.42±2.44%, .73±3.56% in transportation cans while 30.63±4.17% was noted in selling points as shown in Fig 2. The dairy farms had very few milk adulteration activities, whereas milk samples being transported and sold in retail shops had the highest number of fraud attempts.

According to statistical analysis (LSD, 0.05), milk samples collected from transportation cans and sale points included considerably more extraneous water than samples taken from milk storage tanks at dairy farms in five different areas of the city.

Milk specific gravity

Milk samples from three locations (Storage Tanks, Transportation Cans and Selling Points) in each district were analyzed for their average specific gravity. The specific gravity of milk samples collected from dairy farm's storage tanks in district East was recorded as 1.028, followed by transportation cans 1.028 and 1.025 in selling point milk samples.

The specific gravity of milk samples collected from dairy farm's storage tanks in district West was calculated as 1.029, 1.026 in transportation cans while 1.020 was noted in selling points samples. Similarly, 1.031 specific gravity was calculated in the milk samples collected from the storage tanks in district North, 1.026 was recorded in the transportation cans samples and 1.023 was noted in the selling points milk samples. The specific gravity of milk samples collected from dairy farm's storage tanks in district South was recorded as 1.024 followed by milk samples collected from transportation cans 1.021 and 1.017 in selling point milk samples. While, 1.029 was noted in milk samples collected from dairy farm's storage tanks, 1.023 in transportation cans and 1.022 in selling points milk samples collected from district Central (Table 1).

Milk pH values

The average pH of milk samples collected from dairy farm's storage tanks in district East was recorded as 6.88+0.21, 6.85±0.32 was noted in transportation cans samples and 8.85±.0.02 in selling point samples. The pH value of milk samples collected from dairy farm's storage tanks in district West was documented as 6.93±0.30, 6.88±0.026 in transportation cans while 8.89±0.20 was noted in selling points samples. Likewise, 6.87±.031 pH was recorded in the milk samples collected from the storage tanks in district North, 7.91±0.04 was recorded in the transportation cans samples and 8.92±0.023 was noted in the selling points samples. The pH of milk samples collected from dairy farm's storage tanks in district South was recorded as 6.93±0.024 followed by samples collected from transportation cans 6.98±0.036 and 6.90±0.017 in selling point samples. While, 6.90±0.029 was noted in milk samples collected from dairy farm's storage tanks, 6.88±0.023 in transportation cans and 6.93±0.022 in selling points milk samples collected from district Central (Table 1).

Table 1: Raw milk pH, specific gravity, freezing point and % of added water.

Districts	pH			Specific gravity			Freezing points* (C°)			% of added water		
	Sampling spots											
	Storage tanks	Shipment cans	Selling points	Storage tanks	Shipment cans	Selling points	Storage tanks	Shipment cans	Selling points	Storage tanks	Shipment cans	Selling Points
Control	6.4-6.8			1.028 -1.032			(-0.522) to (- 0.540)					
East	6.88±0.14	6.85±0.03	6.85±0.30	1.028±0.06	1.028±0.02	1.025±0.03	-0.531±0.43	-0.496±0.05	-0.340±0.04	10.23±2.13	16.3±4.65	24.60±5.74
West	6.93±1.03	6.88±0.08	8.89±0.42	1.029±0.01	1.026±0.01	1.20±0.05	-0..503±0.34	-0.502±0.14	-0.362±0.03	14.23±3.56	22.15±6.16	32.50±7.86
North	6.67±1.07	6.91±0.1	6.92±0.64	1.031±0.04	1.026±0.04	1.023±0.04	0.478±0.47	-4.57±0.23	-0.340±0.07	13.32±5.32	21.74±6.52	32.37±6.76
South	6.93±1.12	6.95±1.04	6.98±0.52	1.024±0.10	1.021±0.06	1.017±0.04	4.95±0.68	-0.396±0.13	-0.390±0.14	18.36±4.78	32.6±5.87	44.52±7.36
Central	6.90±1.03	6.88±0.06	6.93±0.46	1.029±0.12	1.023±0.05	1.022±0.07	-0.490±0.65	-0.410±0.02	-0.386±0.032	12.42±6.43	18.73±6.56	30.60±6.72
Mean	6.862±1.05	6.90±26	7.31±0.46	1.028±0.06	1.024±0.03	1.017±0.04	-0.452±0.51	-0401±0.1	-0.360±0.06	13.71±4.43	22.30±6.0	32.91±6.82
SE±	0.109	0.03	0.89	0.02	0.04	0.079	0.010	0.013	0.013	2.99	6.22	7.23

*Indicates the mean valve of minimum and maximum range of freezing point according to the formula.

Table 2: Freezing point of milk samples collected from different locations at Karachi.

Descriptive measures	Freezing points of milk (°C)				Significance	
	Control	Dairy farm's storage tank	Milk transportation Cans	Dairy shops	P-value	LSD (0.05) ±SE
Minimum	-0.540	-0.460	-0.411	-0.370	0.012	0.0399±0.0212
Maximum	-0.522	-0.445	-0.392	-0.351		
Mean*	-0.531	-0.452	-0.401	-0.360		
SE±	0.012	0.010	0.013	0.013		

*Means with different letters in same row varied significantly from one another.

Milk freezing point

The average freezing point of control milk was recorded as -0.535±0.027°C. While the freezing point of milk samples collected from dairy shops, transportation cans and milk storage tanks at dairy farms was noted as -0.360±0.013°C, -0.401±0.013°C and -0.452±0.012°C, respectively, as shown in Table 3. Due to the adulteration of water in milk, sample milk's freezing point differed from control milk and was closer to water's zero freezing point. No significant difference (P>0.05) was found in the freezing point of milk samples obtained from transportation cans and dairy stores, according to the least significant difference (LSD, 0.05) of the mean test (Table 2).

Impacts of extra extraneous water on milk specific gravity and pH

Data reveled that extent of extraneous water have strong impacts on the milk specific gravity, the correlation of added water and its impact on its specific gravity is strongly inversely proportional (r = -0.99) to each other. By increasing the water concentration in milk its specific gravity decreases as at 10% extraneous water in milk showed specific gravity of 1.032, 20% extraneous water showed 1.029 specific gravity, 1.022 and 1.017 specific gravity was recorded in milk samples have 30 and 40% extraneous water respectively. Extent of extraneous water usually do not have any significant impact on milk pH value. The correlation between the extent of extraneous water and milk pH is by (r = 0.42). It means the correlation is not strong between these two values. The pH value does not change drastically by increasing the water content in milk (Table 3).

Milk pH as a marker for adulteration detection

It was also investigated how will milk pH worked as a potential indicator for adulteration detection. In situations when the adulterants are either basic or acidic in nature, it is quite practical to gauge the purity of a milk sample from its pH. The values from 6.4-6.8 is the standardized range of pure milk. As urea were detected in 34% milk samples, the average pH value of the same milk showed 8.3±0.23 value, it means that urea has shifted the pH value toward the weak basic range. According to the data detergent were detected in 31% milk samples, it has dramatically increased the milk pH to 9.5±0.15 (basic range). Cane sugar was recorded in 9% milk samples and its pH value was recorded (5.5±0.34) which falls in the acidic range of pH balance. Starch and sodium chlorite was detected in 9% (pH = 6.7±0.07) and 8% (pH = 6.8±0.24) milk samples respectively and the pH values of these samples were found in neutral range. Carbonate was found in 5% milk samples, the pH of these samples showed strong basic nature with pH (9.6±0.03) value. Hypochlorite was detected in 3% milk samples, the average samples showed pH (8.1±0.22) value indicating the weak base range on the pH balance. Formalin was detected in only 1% milk samples. This chemical adulterant affectedly changed the pH value from control range to acidic range (pH = 4.3±0.37). Further, it was noticed that milk samples adulterated with chemical adulterants showed strong shift in specific gravity from control range (Table 4).

According to studies, water is the primary adulterant that is regularly used to adulterate milk in a number of the

Table 3: Percentage of extraneous water and its impact on milk specific gravity and pH values.

% of extraneous water in milk	Physical characteristics	
	Specific gravity	pH
Control	1.032	6.78
10	1.029	6.90
20	1.026	6.93
30	1.022	6.89
40	1.017	6.96
Mean	1.0235	6.92
SE±	0.0051	0.0316

Table 4: Various adulterants effects on milk pH and specific gravity.

	Physical characteristics	
	pH values	Specific gravity
Control	6.40-6.80	1.032
Urea	8.3±0.23	1.34
Caustic Soda	9.5±0.15	1.30
Cane Sugar	5.5±0.34	1.59
Starch	6.7±0.07	1.15
Sodium Chlorite	6.8±0.24	1.00
Carbonate	9.6±0.03	1.34
Hypochlorite	8.1±0.22	1.20
Formalin	4.3±0.37	1.11

world's poorer nations (Barham *et al*., 2014). Comparatively, our findings demonstrated that the milk samples gathered from various places in the southern part of the province of Sindh had deteriorated extensively with contaminated excess water (Memon *et al*., 2018). According to freezing point analysis retail shops samples were adulterated more, followed by transportation cans samples and least extent of extraneous water were recorded in the milk samples collected from dairy's farm milk storage (Barham *et al*., 2014; Barham *et al*., 2015a). Similar observations have been made, showing that milk sold in dairy shops had a remarkably greater level of extraneous water than transportation cans and dairy farm's storage tanks (Barham *et al*., 2015b).

The presence of cane sugar, urea, formalin, sodium chloride, starch, carbonate and hypochlorite was detected in milk samples taken throughout the summer from various places. During the current study, a variety of adulterants were found in raw milk available in Karachi. A significant percentage of milk samples from all of Karachi's districts contained detergents, urea, sodium chloride and cane sugar (Iqbal, 2017; Barham *et al*., 2018). Data showed that the milk sold at these locations was routinely handled improperly from milking until it reached the customer (Iqbal, 2017). It was shown that milk samples taken from shops and shipping cans had a significantly greater amount of adulteration than milk samples taken from dairy farms (Barham *et al*., 2015b). Urea was found in 34% of the milk samples among the total positive tests. Urea is added to milk because it is less expensive, more readily available and a good source of nitrogen. It is added to milk to increase its whiteness, consistency and shelf life as well as to standardize the nonfat solid content (Iqbal, 2017). The second instance was the adulteration of milk using detergent.

Eight samples in district East, six in districts West and North and four in district South all tested positive (Barham *et al*., 2014). In Pakistan, chemical detergents are frequently added to milk to give it a foamy appearance (Iqbal, 2017). It's interesting to note that the intermediaries attempt to reduce the dilution by increasing the amount of solids in the milk or by using chemicals to hide the effect of water dilution (Memon *et al*., 2018). Data revealed that cane sugar was identified in some milk samples; the majority of these samples were taken from district North (Barham *et al*., 2015b; Iqbal, 2017). Starch was also detected in milk samples, however the district South had the highest percentage of starch-adulterated samples relative to the other zones (Barham *et al*., 2018). The milk dealers in Pakistan also regularly use sodium carbonate and bicarbonate to balance the pH and acidity of milk. Carbonate was detected in milk samples collected in each West and Central districts (Barham *et al*., 2014). Hypochlorite adulterated milk samples were only detected in district West. People who already have high blood pressure are gradually poisoned by the practice of adding caustic soda to milk, which already contains salt. Only few milk samples from the area south were found to contain formalin. Formalin, a milk preservative used to extend shelf life, not only reduces the nutritional content of milk but is also cancer-causing (Barham *et al*., 2018; Memon *et al*., 2018).

The milk samples collected from farm storage tanks for the current study had a pH value that was within the normal range. The pH level of the other milk samples, which were taken from shipping containers and dairy stores, was high in excess of the normal range. Although, the adulterant positive samples showed dramatically shift in the pH value according to its acidity or acidity nature. Further, these adulterations also alter the sample specific gravity (Memon *et al*., 2018). The freezing point of the samples differed from that of the control milk because water in milk was adulterated and they goes towards the water's freezing point of zero. Extra water added to milk will have a negative impact on its freezing point and nutritional value (Barham *et al*., 2014; Barham *et al*., 2015a). The average specific gravity of milk samples collected from milk storage tanks on dairy farms was within the acceptable range for control milk. Additionally, milk samples taken from shipping containers and dairy shops have lower specific gravities than is typical (Barham *et al*., 2015a; Barham *et al*., 2020).

CONCLUSION

Raw milk supply chain, from the point of production to consumption in Karachi operates through informal channels. Milk dealers actively engaging in adulteration practices especially milk retailers were found to involve more, followed by milk collectors and producers. In various milk samples, particularly collected from transportation cans and retail shops were found frequently adulterated with exogenous water and other adulterants. Chemical adulteration strongly shifts pH and milk specific gravity from the normal or control range, while addition of water in milk have adverse effect on milk specific gravity and have minimum impact on milk pH values.

Overall, Karachi's milk quality isn't particularly poor, but if the situation isn't handled carefully, it might harm both the dairy industry's finances and the health of its customers in near future, due to inadequate monitoring and law enforcement, the problem milk adulteration is worse in underdeveloped countries.

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Suggestion for consumers

- Always prefer to purchase milk from a reliable license/ registered business source.
- Avoid where there is no cold chain.
- Check for off-taste, discoloration, bad odors, abnormal or damaged packaging appearance while purchasing milk.
- Milk slip test - Put a drop of milk on a polished vertical surface. If it stops or flows slowly, leaving a white trail behind, it is pure milk. Milk mixed with water or other agents will flow down immediately without a trace.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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