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## Physiochemical characterization, water, starch and beta-lactam adulteration in dairy in Algeri

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### Abstract

Milk is a staple food consumed by billions of people worldwide. In Algeria, milk is particularly valued not only for its nutritional benefits but also because it is heavily subsidized, making it an affordable source of protein. However, various forms of adulteration often compromise the quality of milk. In this way, this study investigates the physico-chemical quality and adulteration practices in raw cow's milk in Algeria.

The analyses concerned milk arrival temperature (AT°), pH, titratable acid (TA), specific gravity (Sp.g), fat, total solids (TS), solids-non-fat (SNF), water, starch and betalactam adulteration. The results show an increased and large variability of AT° ( $13.84\text{ }^{\circ}\text{C} \pm 5.41$ ). All samples had a pH lower than the normal pH of fresh milk with a mean of  $6.44 \pm 0.072$ , despite a normal acidity ( $16.64\text{ }^{\circ}\text{D} \pm 0.712$ ). A mean specific gravity (Sp.g) and a mean fat content ( $1.0288\text{ g/mL} \pm 0.0011$  and  $32.24\text{ g/L} \pm 1.72$  respectively), far from Algerian norms; combined with the high level of milk adulterated by water (40%), an acceptable content of SNF ( $8.51\% \pm 0.269$ ) with a weakness in TS ( $11.74\% \pm 0.318$ ). No sample was found to be adulterated with starch. Using the Beta Star test, betalactam residues were found in 22.22% of the samples.

**Keywords:** Raw milk, Physico-chemical quality, Milk fraud.

## 1. Introduction

Milk is one of the most widely consumed foods globally. Over six billion people consume milk and milk products, and between 750 and 900 million people live in dairy-farming households (FAO, 2010). In Algeria, milk is particularly popular for two reasons. First, it is a vital source of high-quality protein, fat, carbohydrates, vitamins, and minerals, which are essential for children's growth and adults' health (Faraz *et al.*, 2013; Ali *et al.*, 2021). Milk can also serve as a cost-effective alternative to other expensive products like meat (Zeghilet *et al.*, 2017). Second, the Algerian government subsidizes milk, making it the most affordable source of protein (Lazereg *et*

*al.*, 2020). This is why Algeria is the largest milk consumer in the Maghreb, with an average consumption of 157 kg per year (Zeghilet *et al.*, 2017).

However, milk is often subject to adulteration, such as the addition of water, which reduces its dry matter content and alters its physico-chemical properties. Starch is sometimes added to increase the milk's consistency and viscosity. Another serious issue is the presence of antibiotic residues, which can affect the dairy industry and human health (Layada *et al.*, 2016). Adulteration is particularly problematic in developing and underdeveloped countries due to inadequate monitoring and weak law enforcement (Azad and Ahmed, 2016).

In Algeria, there is limited data on the physico-chemical quality and adulteration of raw cow's milk. Therefore, the main aim of this study is to report and describe these practices in the Algerian dairy industry.

## 2. Materials and Methods

### Sampling

This study was conducted in a dairy industry setting. Forty-five samples of raw cow's milk were collected from different storage tanks, with the milk sourced from various farms in the region. Sampling took place at the beginning of the warm season, specifically from late April to mid-June 2020. The milk was collected in sterile containers and transported to the Quality Service Laboratory, which is situated about ten minutes from the collection vehicle's parking area. The samples were analyzed within fifteen minutes of collection to ensure their integrity and accuracy.

### Physicochemical analysis

**The temperature** of the samples is recorded using a thermometer type Hanna Instrument, HI98509.

A **pH** meter is used to determine the hydrogen ion activity of the samples. (Microprocessor Meter, pH 211, Hanna Instrument). Before use, the meter is calibrated with buffer solutions of pH 4.0 and 7.0. TA.

**Acidity**, expressed in degrees Dornic ( $^{\circ}\text{D}$ ), is determined by titrating a 10 ml sample of milk with a N/9 sodium hydroxide (NaOH) solution to raise the pH of the sample to approximately 8.4, in the presence of phenolphthalein 1% as an indicator. The end of the titration is reached when a pink color appears in the milk and persists for approximately ten seconds. The volume (in milliliters) of the sodium hydroxide solution used is noted at the end. To convert the result into degrees Dornic ( $^{\circ}\text{D}$ ), the recorded volume is multiplied by 10.

**The density** of the milk was determined using a thermo-lactodensimeter (model Laktodensimeter, Milch g/L T $^{\circ}$  20 $^{\circ}\text{C}$ , 45 mm, Mbl Obin, Funk Gerber Berlin, 1.020-1.040). The reading is taken at 20 $^{\circ}\text{C}$ . If the milk temperature deviates from 20 $^{\circ}\text{C}$  the following correction formula is applied: Corrected Density = Reading Density + 0.2  $\times$  (Milk Temperature–20 C) (AOAC, 1990).

Specific gravity (Sp.g), expressed in g/mL, was calculated using the following formula:

$$\text{Sp.g} = (\text{CLR}/1000) + 1,$$

where CLR = corrected lactometer reading or density (AOAC, 1990).

**Fat content** was determined using the Gerber acid-butyrometric method. In this method, 11 ml of the milk sample is mixed with 10 ml of concentrated sulfuric acid and 1 ml of iso-amyl alcohol in a butyrometer and centrifuged at 1200 rpm for 5 minutes. The fat content is read directly from the graduated scale on the Gerber butyrometer (Guiraud, 2012).

**Milk Total Solids (TS)** and **Solid-Not-Fat (SNF)** were calculated using Richmond's formula (Vishweshwar and Krishnaiah, 2005):

$$\% \text{ TS} = 0.25 \times D + 1.2 \times F + 0.66$$

$$\% \text{ SNF} = 0.25 \times D + 0.2 \times F + 0.66$$

Where D = Density reading at 20°C, and F = % Fat.

**Added Water (AW)** was estimated using the following formula:

$\% \text{ AW} = (S - s) \times 100$ , where S = standard % SNF (8.5% for cow's milk), and s = % SNF of the milk sample (Singh and Gandhi, 2015).

**Starch adulteration** was screened using an iodine solution. One to three drops of 1% iodine solution (prepared by mixing 1 g of dehydrated iodine with 2 g of dehydrated potassium iodide in 100 ml of distilled water) were added to 3 ml of the milk sample. The appearance of an intense blue-violet color indicated the presence of starch (JOCE, 1994).

**Beta-lactam residues** in milk were screened using the Beta-star Screening Kit (Neogen Corporation, USA). The test is validated for use with raw and pooled cow's milk samples. A 0.2 ml sample of milk is added to the reagents in the vial, which is then stoppered, inverted, and swirled to dissolve all solids. After dissolution, the vial is incubated in a block heater at 47.5°C for 3 minutes. The dipstick is then added and the incubation continued at the same temperature for 2 minutes. The color intensity of the test bands is visually compared to the reference band. If the test band is weaker than the reference band, the sample is considered positive for beta-lactam residues (Gustavsson and Sternesjö, 2004; Navrátilová, 2008).

### Statistical Analysis

Statistical analysis of the collected data was performed using Minitab 16.1.1.0 (Minitab, 2010) and IBM SPSS (Statistical Package for the Social Sciences) version 20. Descriptive statistics were used to summarize the data, and correlation analysis was applied to investigate relationships between different variables.

## 3. Results and discussion

### Physico-chemical analyzes

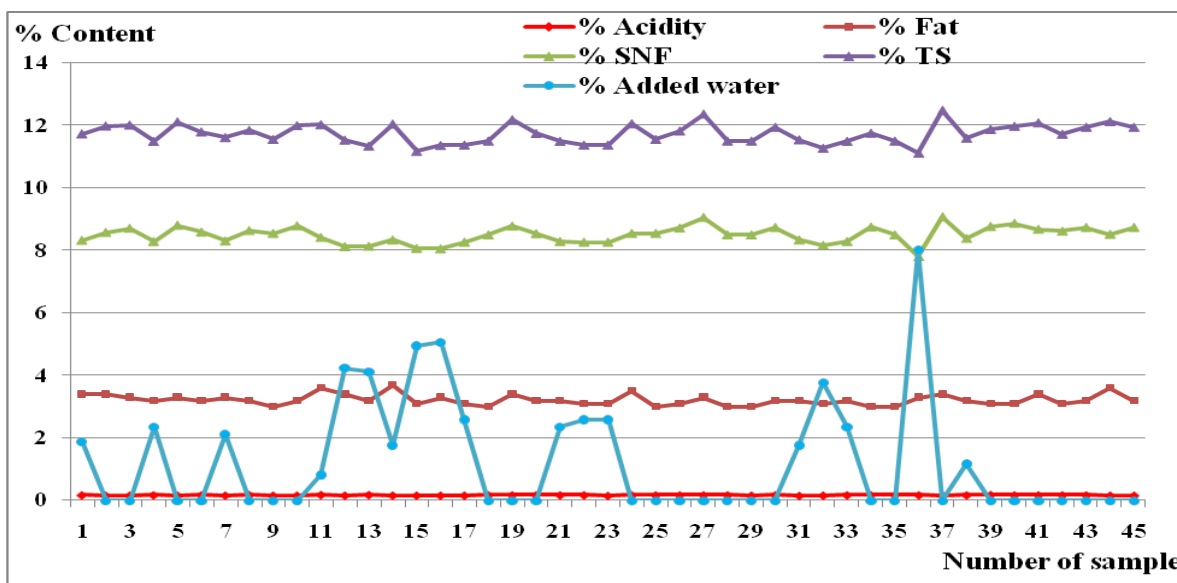
The physico-chemical analyzes results of milk samples are given in Table 1 and Fig 1.

**Table 1.** Comparison between physicochemical quality of samples and standards using one sample t- Student test.

Parameter	Standards	Min	Max	Mean	SD	t-values	p- values	Significance	95% CI
AT° (°C)	06	05	25	13.84	5.41	9.72	0.000	**	(12.218; 15.471)

<b>pH</b>	6.6-6.8	6.18	6.53	6.44	0.072	-14.83 to -33.40	0.000	**	(6.4185; 6.4619)
<b>Acidity (°D)</b>	Max 18	15	18	16.64	0.712	6.07 to -12.77	0.000	*	(16.431; 16.858)
<b>Sp.g (g/mL)</b>	1.030-1.034	1.026	1.031	1.0288	0.0011	-7.24 to -31.91	0.000	**	(1.02850; 1.02915)
<b>Fat (g/L)</b>	Min 34	30	37	32.24	1.72	-6.84	0.000	**	(31.727; 32.761)
<b>% TS</b>	12	11.12	12.49	11.74	0.318	-5.57	0.000	**	(11.6404; 11.8316)
<b>% SNF</b>	8.5	7.82	9.09	8.51	0.269	0.29	0.775	*	(8.4306; 8.5925)

Min: minimum, Max: maximum, \* No significant difference, \*\* significant difference, P<0.05, CI: Confidence Interval.



**Figure 1:** Acidity, Fat, SNF, TS, and AW variations among analyzed samples.

This study provides a detailed analysis of the physicochemical quality of milk samples, comparing them with established standards using a one-sample t-test. The results highlight significant deviations in several key parameters, indicating potential quality issues and areas for improvement in milk handling and processing practices.

**Temperature (AT°)**

The average temperature (AT°) of the milk samples was found to be 13.84°C ± 5.41, significantly higher than the recommended standard of 6°C (t = 9.72, p < 0.000). This finding suggests inadequate cooling practices during milk collection and transportation. As noted by Al-Qadiri *et al.* (2008) high storage temperatures can accelerate microbial spoilage, leading to rapid deterioration of milk quality. Keeping milk in poor conditions for more than 4 hours promotes microbial growth (Yuen *et al.*, 2012). Indeed, if raw milk is not cooled immediately after milking, lactic acid bacteria will multiply within 2-3 hours (Kurwijila, 2006). The lack of proper cooling infrastructure, such as isothermal tanks, likely contributes to this issue, as supported by findings from Kouamé-

Sina *et al.* (2010). The results clearly indicate a lack of a proper cold chain in this sector; the standards are not met by 86.67% of the collectors. Edima *et al.* (2013) reported an even worse situation in Cameroon. Our average milk arrival temperature (AT°) is similar to that found at dairy farms by Srairi and Hamama (2006) in Morocco (13.4°C), but much better than the temperature of milk intended for sale studied by Kouamé-Sina *et al.* (2010) in Ivory Coast (31.9°C).

### **pH**

The pH values ranged from 6.18 to 6.53, with an average of  $6.44 \pm 0.072$ . Only ten samples had a pH value close to the normal range for fresh milk. These values fall outside the normal range of 6.6-6.8 ( $t = -14.83$  to  $-33.40$ ,  $p < 0.000$ ), indicating potential spoilage or contamination. Labioui *et al.* (2009) report similar results (6.55). Fresh milk generally has a slightly acidic to neutral pH, and deviations can be indicative of bacterial activity or the addition of substances to prolong shelf life (Anderson *et al.*, 2011; Javaid *et al.*, 2009).

### **Acidity (°D)**

The acidity of the milk samples varied from 15 to 18°D, with an average of  $16.64^{\circ}\text{D} \pm 0.712$ , which is within the acceptable range but still shows significant variation ( $t = 6.07$  to  $-12.77$ ,  $p < 0.000$ ). The natural acidity of milk is crucial for processing, and deviations can affect product quality. As indicated by Karthikeyan *et al.* (2012), proper management of acidity levels is essential for maintaining milk's suitability for processing and must be between 15 and 18°D. In this study, 86.67% of samples having an acidity between 16 and 17°D, 8.89% having 18°D, and only 4.44% having an acidity of 15°D. Labioui *et al.* (2009) report similar results (16.75°D). These authors explain that acidity and pH values depend mainly on the content of casein, mineral salts and ions.

### **Specific Gravity (Sp.g)**

The specific gravity of the milk samples average is  $1.0288 \text{ g.mL}^{-1} \pm 0.0011$ , slightly below the standard range of 1.030-1.034 ( $t = -7.24$  to  $-31.91$ ,  $p < 0.000$ ). FAO (1995) and Algerian standards (JORADP, 1993) recommend Sp g values of 1.028 to 1.033 g/mL and 1.030 to 1.034 g/mL, respectively. This deviation could be due to adulteration with water, as specific gravity is closely linked to milk's solid content, including fats and proteins (Labioui *et al.*, 2009). In the present study, the average Sp g is similar to that found by Aggad *et al.* (2009) in western Algeria (1.0290 g/mL), but slightly lower than Labioui *et al.* (2009) in Morocco (1.0297 g/mL). The study by Srairi and Hamama (2006) also noted variations in specific gravity due to such adulteration practices.

### **Fat Content**

The fat content ranged from 30 to 37 g.L<sup>-1</sup>, with an average of  $32.24 \text{ g.L}^{-1} \pm 1.72$ , below the minimum recommended standard of 34 g.L<sup>-1</sup> ( $t = -6.84$ ,  $p < 0.000$ ). Such mean value is very similar to that reported in the south of Tunisia (32.5g/L) (Sboui *et al.*, 2009), but it stay slightly good to that reported during the same year in Morocco (31.4g/L) (Labioui *et al.*, 2009) and very weak compared to that reported in Serbia ( $4.03 \pm 0.34$ ) by Milićević *et al.* (2022). Fat content is a critical determinant of milk's nutritional and processing quality. Factors such as breed differences and feeding practices can influence fat levels, as described by Kurwijila (2006) and

Humski *et al.* (2018). Additionally, factors like climate, lactation stage and diet significantly impact milk fat content (Labioui *et al.*, 2009).

The quality and characteristics of forage, particularly its physical structure and energy content per unit of dry matter, play a critical role in determining fat levels in milk (Jasińska *et al.*, 2010). Environmental conditions, such as heat, can lead to reduced saliva production in cows, lowering rumen pH and subsequently reducing acetic acid production, which is essential for milk fat synthesis (Humski *et al.*, 2018). For instance, during hot summers, cows may produce more diluted, low-fat milk (Khaskheli *et al.*, 2005).

Seasonal variations also affect milk fat percentage. It tends to be lowest from April to July in both pasture and confinement systems (Wyss *et al.*, 2011). On the other, high-fat milk is common at the start of lactation due to the mobilization of fat reserves, but this study focused on cows in their 2nd to 3rd month of lactation, a period when fat content typically decreases (Woldecherkos and Yitayal, 2003). Furthermore, factors like high somatic cell counts (SCC) and water adulteration can also reduce milk fat content (Mikulec *et al.*, 2012).

### **Total Solids (TS)**

The total solids content ranged from 11.12% to 12.49%, with a mean of 11.74%  $\pm$  0.318, also below the recommended minimum of 12% ( $t = -5.57$ ,  $p < 0.000$ ). These results are similar to those reported by Kittivachra *et al.* (2006) (11.92%) and Sboui *et al.* (2009) (11.94%), but lower than those observed by Siboukeur and Siboukeur (2012) (12.80%). Total solids include fats, proteins, lactose and minerals, all of which contribute to the nutritional value of milk, which varies according to animal diet and climate, as noted by Dănuț-Mocanu *et al.* (2011).

### **Solids-Not-Fat (SNF)**

The SNF content ranged from 7.82% to 9.09%, with an average of 8.51%  $\pm$  0.269, meeting the minimum standard of 8.5% but showing significant variability ( $t = 0.29$ ,  $p = 0.775$ ). SNF is crucial component for cheese and yogurt production, as it affects yield and texture. The results align with findings of studies by Donkor *et al.* (2007), Bille *et al.* (2009) and Milićević *et al.*, (2022) who reported similar ranges in their analyses. Generally, as fat content increases, TS and SNF levels increase while Sp g decreases (Bille *et al.*, 2009).

### **Milk fraud**

Adulteration of water and starch, as well as contamination by beta-lactam residues are reported in Table 2.

**Table 2.** Results of adulteration milk by water, starch and Beta-lactams residues.

<b>Adulterant</b>	<b>Number of samples</b>	<b>Positive samples</b>	<b>Negative samples</b>
<b>Water</b>	45	40 %	60 %
<b>Starch</b>	45	0.0 %	0.0 %
<b>Beta-lactams residues</b>	45	22.22%	77.78%

The results in Table 2 reveal significant issues with milk adulteration and contamination, which have an impact on the quality and safety of the milk in the samples studied.

**Water Adulteration**

The data indicates that 40% of the 45 milk samples tested were adulterated with water. This high rate of adulteration aligns with findings from other studies in various regions. For instance, in Ghana, Donkor *et al.* (2007) reported similar issues with water adulteration in milk, although the rate was lower than that observed in Algeria. Conversely, a study in India by Nirwal *et al.* (2013) and Pakistan by Shaikh *et al.* (2013) found an even higher rate of water adulteration, underscoring the global nature of this problem. Adding water to milk, while increasing its volume, significantly dilutes its nutritional value and poses health risks, particularly if contaminated water is used (Gawali, 2021). It should be noted that Cow's milk is often adulterated because it is widely produced and water is cheaper (Borková and Snášelová, 2005), so control must be stringent; such as in USA, they reject raw milk with 1% or more added water (Popescu and Angel, 2009).

**Starch Adulteration**

None of the samples tested positive for starch adulteration. This result is consistent with studies conducted in Bangladesh and Faisalabad, where Bellal and Sima (2013) and Faraz *et al.* (2013) also found no evidence of starch adulteration in milk. The absence of starch adulteration could be due to its higher cost and the difficulty in homogenizing starch within milk, making it easily detectable by consumers (Ahmad, 2009). Additionally, milk collectors may lack familiarity with starch adulteration techniques.

**Beta-lactam Antibiotic Residues**

The presence of beta-lactam residues in 22.22% of the samples is a significant concern (table 2). Beta-lactams, including penicillin G, are commonly used in veterinary medicine, especially for treating mastitis in dairy cows (Ardıç and Durmaz, 2006; Zeghilet *et al.*, 2022). The detection of these residues suggests improper use and insufficient withdrawal periods before milk collection, leading to contamination. This finding is comparable to contamination rates found in Turkey (21.3%) by Ardıç and Durmaz (2006) and lower than those reported in Pakistan (36.5%) by Khaskheli *et al.* (2008) and in Kosovo (50.4%) by Ibraimi *et al.* (2013). The presence of antibiotic residues not only poses a health risk to consumers, potentially causing allergic reactions and antibiotic resistance, but also interferes with dairy product processing by affecting starter cultures and fermentation processes (Pogurschi *et al.*, 2015; Valença *et al.*, 2021).

**Correlation strength between the different variables**

The data of correlation strength among AT°, pH, Acidity, Sp.g, Fat, SNF, and percentage of TS is given in Table 3.

**Table 3.** Data of correlation strength among AT°, pH, Acidity, Sp.g, Fat, SNF, and percentage of TS.

Variables	AT° (°C)	pH	Acidity (°D)	Sp.g (g/mL)	Fat %	SNF%	TS%
A T° (°C)	1						

<b>pH</b>	-0.036	1					
<b>Acidity (°D)</b>	-0.056	0.032	1				
<b>Sp.g (g/mL)</b>	0.432	-0.059	0.142	1			
<b>Fat %</b>	-0.084	-0.163	-0.057	-0.137	1		
<b>SNF%</b>	0.426	-0.080	0.136	0.992	-0.011	1	
<b>TS%</b>	0.315	-0.156	0.084	0.766	0.532	0.841	1

Table 3 presents the data of correlation strength among various physicochemical parameters of milk: Arrival temperature (AT°), pH, acidity (°D), specific gravity (Sp.g), fat percentage, solids-not-fat (SNF) percentage and total solids (TS) percentage. This analysis is essential for understanding how these variables interact and influence milk quality.

### Arrival Temperature (AT°)

The data indicates that AT° has a weak negative correlation with pH (-0.036) and acidity (-0.056), suggesting that higher milk temperatures slightly decrease milk pH and acidity. However, AT° shows a moderate positive correlation with specific gravity (0.432), SNF (0.426), and TS (0.315). This suggests that as milk temperature increases, the specific gravity, SNF, and TS of milk also tend to increase. This could be attributed to the increased evaporation of water content from milk at higher temperatures, concentrating other components.

### pH

The pH of milk has negligible to weak negative correlations with all other parameters except acidity, with which it has a very weak positive correlation (0.032). This indicates that the pH of milk is relatively stable and does not significantly influence or is influenced by other physicochemical properties within the tested range. Fresh milk typically has a pH of around 6.6 to 6.8, and deviations from this range can indicate microbial activity or adulteration (Anderson *et al.*, 2011). TA and pH results are not greatly correlating to each other and this could be attributed to addition of water, ice or chemical preservative to extend shelf life of milk (Javaid *et al.*, 2009), or because there's not a direct relation between TA and pH linked to the buffering capacity of milk (Tourette *et al.*, 2002).

### Acidity

Acidity shows very weak correlations with all other parameters, with the highest being with specific gravity (0.142) and SNF (0.136). This suggests that while acidity is an important quality parameter, its direct influence on other physicochemical properties is limited. The acidity of milk, expressed in degrees Dornic (°D), is typically between 15°D to 18°D for raw milk, indicating its suitability for processing (Karthikeyan *et al.*, 2012).

### Specific Gravity (Sp.g)

Specific gravity shows a very high positive correlation with SNF (0.992), indicating that changes in the specific gravity of milk are almost entirely due to changes in its SNF content. It also shows a strong positive correlation with TS (0.766), further emphasizing the influence of non-fat components on milk density. Specific gravity is an

essential indicator of milk adulteration; a lower than normal value can indicate dilution with water (Labioui *et al.*, 2009).

### **Fat content**

Fat percentage exhibits weak negative correlations with AT° (-0.084), pH (-0.163), acidity (-0.057), and specific gravity (-0.137). Interestingly, it has a moderate positive correlation with TS (0.532), indicating that fat content contributes to the total solid content of milk but does not strongly influence or get influenced by other parameters. Fat content is a key quality indicator and is susceptible to variations due to feeding practices and breed of cattle (Kurwijila, 2006).

### **Solids-Not-Fat (SNF) and Total Solids (TS)**

SNF shows a very strong positive correlation with specific gravity (0.992) and TS (0.841), highlighting its significant role in determining these parameters. TS, which includes both fat and SNF, is also moderately positively correlated with fat percentage (0.532). This underscores the composite nature of TS as a critical measure of milk's overall quality and nutritional value. High SNF content often reflects good feeding practices and proper milk handling (Dănuț-Mocanu *et al.*, 2011).

## **4. Conclusion**

This experimental study shows that the milk collection process negatively affects milk quality. This is mainly due to hawkers acting as intermediaries between farmers and pasteurizing centers. These centers often accept milk that does not meet standards for fat content, specific gravity, and total solids content, and payment for the milk does not reflect its quality. Additionally, the milk often arrives at the centers at a high storage temperature, diluted with water, and contaminated with beta-lactam antibiotic residues. The competition between nearby centers to maximize volume, driven by the desire for higher subsidies, exacerbates the problem.

Furthermore, the presence of antibiotics poses a significant risk to the dairy processing industry. Therefore, it is essential to strengthen inspection programs to closely monitor the treatment records of dairy cows and ensure the safety and processability of the milk.

### **Conflict of interest**

Authors declared no conflict of interest exist.

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