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Assessment of microbiological and physicochemical quality of raw milk sold informally through social media

Avaliação da qualidade microbiológica e físico-química do leite cru comercializado informalmente por rede social

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Abstract: This study evaluated the quality of raw milk sold informally in Cuiabá, Mato Grosso, Brazil, focusing on the ease of acquisition via social media and the association of this practice with fraud and serious quality issues. Eighty liters of raw milk were acquired from four distinct producers via social media, totaling 40 samples. The analyses revealed high microbiological contamination, with elevated counts of aerobic mesophilic bacteria (8.63 log_{10} colony-forming units [CFU]/mL), *Enterobacteriaceae* (7.71 log_{10} CFU/mL), total coliforms (4.15 log_{10} most probable number [MPN]/mL), thermotolerant coliforms (3.34 log_{10} MPN/mL), *Escherichia coli* (3.57 log_{10} MPN/mL), and psychrotrophic bacteria (4.41 log_{10} CFU/mL). *Salmonella* spp. was not detected. Physicochemical analyses indicated non-compliance with parameters for fat (15%), protein (20%), lactose (15%), solids non-fat (47.5%), total solids (20%), density (20%), the cryoscopic index (82.5%), and Dornic acidity (60%). Additionally, 70% of the samples exhibited instability in the alcohol-alizarin test, with a pH ranging from 5.58 to 6.12. We identified several frauds—the addition of water, sucrose, hydrogen peroxide, and antimicrobial residues—underscoring the severity of informal trade. Additionally, as part of this study, we investigated the antimicrobial efficacy of different disinfectants commonly used or suggested for sanitation in milk production environments. Among the five disinfectants tested, 2.5% sodium hypochlorite demonstrated the highest antimicrobial efficacy, followed by 3% hydrogen peroxide and benzalkonium chloride. Iodophor at 0.4% and sodium hypochlorite diluted according to the manufacturer's recommendations did not show a significant effect. The results highlight the poor microbiological and physicochemical quality of informally traded raw milk in this region of Brazil, with contamination and fraud posing a substantial sanitary risk to consumers. The results strongly advocate for more rigorous oversight and greater public understanding regarding the risks associated with consuming uninspected milk.

Key-words: hygiene; food microbiology; food fraud; public health; coliforms; bovine milk.

Resumo: Este estudo avaliou a qualidade do leite cru comercializado informalmente em Cuiabá, Mato Grosso, Brasil, com ênfase na facilidade de aquisição via rede social e na associação dessa prática com fraudes e graves problemas de qualidade. Foram adquiridos 80 litros de leite cru de quatro produtores distintos por meio de rede social, totalizando 40 amostras. As análises revelaram elevada contaminação microbiológica, com contagens altas de bactérias aeróbias mesófilas (8,63 log_{10} unidades formadoras de colônia [UFC]/mL), *Enterobacteriaceae* (7,71 log_{10} UFC/mL), coliformes totais (4,15 log_{10} número mais provável [NMP]/mL), coliformes termotolerantes (3,34 log_{10} NMP/mL), *Escherichia coli* (3,57 log_{10} NMP/mL) e bactérias psicrotróficas (4,41 log_{10} UFC/mL). *Salmonella* spp. não foi detectada. As análises físico-químicas indicaram não conformidade em relação aos parâmetros de gordura (15%), proteína (20%), lactose (15%), sólidos desengordurados (47,5%), sólidos totais (20%), densidade (20%), índice crioscópico (82,5%) e acidez Dornic (60%). Além disso, 70% das amostras apresentaram instabilidade no teste do álcool-alizarol, com valores de pH variando entre 5,58 e 6,12. Foram identificadas diversas fraudes — adição de água, sacarose, peróxido de hidrogênio e resíduos de antimicrobianos — evidenciando a gravidade do comércio informal. Adicionalmente, como parte deste estudo, investigou-se a eficácia antimicrobiana de diferentes desinfetantes comumente utilizados ou sugeridos para higienização em ambientes de produção de leite. Entre os cinco desinfetantes testados, o hipoclorito de sódio a 2,5% apresentou a maior eficácia antimicrobiana, seguido do peróxido de hidrogênio a 3% e do cloreto de benzalcônio. O iodóforo a 0,4% e o hipoclorito de sódio diluído de acordo com as recomendações do fabricante não apresentaram efeito significativo. Os resultados evidenciam a baixa qualidade microbiológica e físico-química do leite cru comercializado informalmente nesta região do Brasil, com a contaminação e as fraudes representando um risco sanitário substancial aos consumidores. Esses achados reforçam a necessidade de uma fiscalização mais rigorosa e de maior conscientização pública quanto aos riscos associados ao consumo de leite sem inspeção.

Palavras-chave: higiene; microbiologia de alimentos; fraude alimentar; saúde pública; coliformes; leite bovino.

1. Introduction

Brazilian legislation prohibited raw milk trade in 1969 (1), yet it persists due to cultural factors and beliefs in its superiority. Although research has revealed the illegality and health consequences of raw milk, many consumers remain unaware of these issues. This is further complicated by the unfounded belief that industrialized milk is inferior due to preservatives (2). Other countries show a similar pattern: In rural Turkey, for example, raw milk consumption is related to people with low education and income (3).

Both raw milk and its derivatives can carry pathogens of great importance to public health (4-7). Some of these were responsible for 3,521 cases of dairy consumption illnesses between 2000 and 2021 in Brazil, resulting in 4 deaths (8). The United States experienced 81 raw-milk-associated outbreaks across 26 states between 2007 and 2012, resulting in 979 illnesses and 73 hospitalizations (9). Informal trade also involves adulteration (10,11) and economic issues due to the non-collection of taxes and unfair competition with legalized companies (12). Consequently, we sought to assess the quality of raw milk marketed online by conducting microbiological, physicochemical, and fraud analyses. As a potential strategy to mitigate contamination risks, we examined the effectiveness of commonly used disinfectants in milking hygiene.

2. Material and methods

We conducted this research at LAQUA, the Food Quality Laboratory of the Federal University of Mato Grosso (UFMT), Cuiabá campus, Brazil.

2.1 Sampling

We analyzed a total of 40 raw milk samples, each comprising a 2-L polyethylene terephthalate (PET) plastic bottle, for a total of 80 L. These samples were acquired during the first semester of 2023 from four distinct producers. The producers advertised their raw milk products, along with contact information and pickup addresses, on social media marketplaces, often including photographs of the milk in PET bottles and, in many cases, also of dairy products. Home delivery was also an option, typically for an additional fee. All purchase agreements were finalized via the marketplace's chat function. The time between acquisition and delivery varied among the sellers: Two producers had their product consistently available for pickup, so the researcher collected the product the day after contact. The other two, who had regular customers and made weekly deliveries, delivered the milk directly to the researchers' homes. The price per bottle ranged from R$10.00 to R$16.00. Upon collection, the bottles temperature was approximately 15°C. They were then immediately placed in a cooler and transported to the laboratory for analysis.

2.2 Microbiological analyses

For inoculum preparation, serial dilutions were performed following ISO 6887-1 [13], and three suitable dilutions were selected for cultivation. Microbiological enumeration of mesophilic, *Enterobacteriaceae*, and psychrotrophic bacteria was carried out using the pour plate and spread plate techniques as described by Salfinger and Tortorello [14]. Total and thermotolerant coliforms were quantified using the multiple-tube fermentation technique outlined in ISO 6887-1 [13]. Finally, *Salmonella* spp. detection followed the methodology of the Brazilian Ministry of Health [15].

2.3 Physicochemical analyses

We obtained the density at 15°C by using the Thermolactodensimeter Quevenne and corrected it for temperature. We determined the titratable acidity with a Dornic acidimeter. To analyze stability to 72% ethanol, we performed the alcohol-alizarin test by mixing equal parts of the sample and 72% Alizarin (LABLAC®) and observed color and curd formation. We calculated the total solids (TS) content by combining density and the fat content by using an Ackermann disc [16], and we measured pH with a digital potentiometer following the manufacturer's instructions. An automatic ultrasound analyzer (LACTOSCAN SP®) provided the contents of fat, solids non-fat (SNF), protein, lactose, and salts as well as the cryoscopic index, according to the manufacturer's recommendations.

2.4 Fraud

We screened for urea and alkaline phosphatase by using rapid and semi-quantitative strips for milk adulteration (CAP-LAB®). We detected antibiotic residues with the commercial Eclipse 50 test (CAP-LAB®), which is based on microbial growth inhibition. All these procedures followed the manufacturer's recommendations. For chlorine, hypochlorites, hydrogen peroxide, soda, and starch, we followed the methodology proposed by Tronco [17], and for sucrose, we used the method reported by Marques et al. [18].

2.5 Disinfectant resistance

We acquired disinfectants from commercial brands and designated them "A," "B," "C," "D," and "E," corresponding to the following active principles: 0.4% iodophor; 3% hydrogen peroxide (H_2O_2) benzalkonium chloride, sodium hypochlorite (2.5%), and sodium hypochlorite diluted according to the manufacturer's recommendations for environmental disinfection, respectively. For the analysis, we used the disc-diffusion technique. With a bacteriological loop, we inoculated colonies of mesophilic bacteria, *Enterobacteriaceae*, and total coliforms, previously isolated from the samples, into nutrient broth (NB) and incubated them at 36°C for 24 hours. Then, we applied a swab to spread the inoculum onto nutrient agar plates. Next, we positioned filter paper discs that had been soaked in the disinfectants onto the agar surface. We then placed the plates in an incubator at 36°C for 24 hours, after which we measured the inhibition zone that developed around the discs using a caliper [19,20].

2.6 Statistical analysis

We used R to perform the Tukey test to identify significant differences in microbiological counts between producers and the effectiveness of the tested disinfectants.

3. Results and discussion

3.1 Physicochemical analyses and fraud investigation

Table 1 illustrates the average of the parameters that we analyzed for the samples from each milk producer group.

Table 1. The physicochemical parameters of raw milk commercialized in Cuiabá, Mato Grosso, Brazil.

Parameter	A	B	C	D	Standard[21]
Fat (%)	3.29	4.29	4.59	3.73	3 (Min. %)
Protein (%)	3.32	3.23	2.78	3.08	2.9 (Min. %)
Lactose (%)	4.77	4.87	3.86	4.64	4.3 (Min. %)
Solids non-fat (%)	8.54	8.84	7.67	8.44	8.4 (Min. %)
Total solids (%)	11.49	13.14	12.27	12.17	11.4 (Min. %)
Density at 15ºC	1.030	1.032	1.026	1.030	1.028 to 1.034
Cryoscopy (ºC)	-0.555	-0.587	-0.5445	-0.540	-0.512 to -0.536
Acidity (ºD)	21.93	20.15	16.97	19.8	14 to 18ºD
pH	6.11	6.12	5.58	5.83	6.6 to 6.8

Overall, 60% of the analyzed milk samples had unsatisfactory Dornic acidity (average 19.71°D, legal range 14-18°D). Of these, 95.83% showed high acidity, suggesting inadequate hygiene and elevated transport temperatures, conditions that favor coliform growth and the conversion of lactose into acids and gases [22]. The 29 (72.5%) samples that showed instability to the alcohol-alizarin test and an average pH of 5.83 corroborate this finding. Although pH is not a legal requirement, it is an important parameter because it is directly related to the microbiological quality and thermal stability of milk, typically ranging from 6.6 to 6.8. Analyzing the quality of raw milk sold in Imperatriz, Maranhão, Brazil, Souza *et al.* [23] also observed non-compliance regarding Dornic acidity (35%) and pH (15%), findings that support the low quality of raw milk in other regions of Brazil. The average pH, Dornic acidity, and alcohol-alizarin test results in the raw milk samples highlight the prevalence of acidic milk.

While the average TS in the milk samples met the minimum legal limit (11.4%), 20% of the samples were below this threshold. Additionally, the average SNF was 8.3%, falling short of the 8.4% minimum, with 47.5% of samples below this threshold. These reductions in TS and SNF strongly suggest illegal adulteration, which is likely to increase the volume or conceal degradation and, consequently, endanger consumers. Similar findings of low SNF in raw milk have been reported in other studies [24,25].

The average fat, protein, and lactose contents were within the legal limits, but individual samples showed lower contents (15% for fat, 20% for protein, and 15% for lactose). These low individual values point toward the addition of water to increase the volume or skimming for cream/butter production, practices also noted in other Brazilian studies [26]. Adding whey is another simple and cheap method used by producers on the informal market to increase volume and to dilute solids, but it can be detected by cryoscopy and density analysis [27].

The addition of water increases the cryoscopic index and decreases density. While the average density was 1.030, 20% of the samples were below the legal minimum (1.028-1.034). Moreover, 82.5% of samples had an inadequate cryoscopic index (-0.599 to -0.413°C), with 30.3% suggesting water addition and 69.6% indicating the use of reconstituting agents, such as salt, sucrose, starch, and alcohol, to mask water addition [28]. Our findings parallel those of Trindade *et al.* [29], who, despite not detecting a density below the minimum limit, also found an altered cryoscopic index in all of their samples. Our automatic ultrasound milk analyzer detected water addition in 30% of the samples, ranging from 0.19% to 20.57%. Among these adulterated samples, 83.33% showed an elevated cryoscopic index and 58% had a low density.

While we found no evidence of starch, 7.5% of samples tested positive for sucrose. Of these sucrose-positive samples, one (2.5% of the total) had a normal cryoscopic index (-0.522°C), while two showed a high cryoscopic index (-0.536 and -0.588°C). Given these findings, it is probable that sucrose was not the only substance employed for reconstitution. The high cryoscopic index could also be attributed to the presence of other unmeasured components such as salt and alcohol. Firmino *et al.* [30] examined refrigerated raw milk and found no starch but detected chlorides (36%), sucrose (6%), and even urine (52%). Oliveira and Santos [31] found alkaline substances (80%) and chlorides (16.6%).

Although we did not detect neutralizers, which are fraudulently used to conceal acidity resulting from poor hygiene, we found H_2O_2, an antimicrobial preservative [27], in 20% of the samples. This finding is concerning due the potential of H_2O_2 to damage the gastrointestinal mucosa and to cause gastritis, enteritis, and dysentery [32].

A review of Brazilian food fraud cases between 2007 and 2017 showed milk and derivatives were the most affected, mainly by water adulteration, aligning with this study's findings. Other adulterants include urine, formaldehyde, hydrogen peroxide, and sucrose, among others, but underreporting is common [33]. We detected antimicrobial residues in 7.5% of the samples. Importantly, even in the samples where our tests did not detect residues, some of them could have levels below our detection limit, posing risks of allergic reactions and contributing to the growing problem of antibiotic resistance [34,35].

Alkaline phosphatase was present in all samples. This enzyme is rendered inactive by pasteurization—and indeed serves as a key indicator of its effectiveness [36]. Hence, its presence is a strong indication that none of the milk samples underwent heat treatment.

3.2 Microbiological analyses

3.2.1 Mesophiles

Analyses of raw milk samples from four producers (A, B, C, and D) revealed elevated mesophilic counts (Table 2), with average value of 7.48, 9.12, 8.27, and 9.66 log_{10} colony-forming units (CFU)/mL, respectively (ranging from 4.30 to 11.53 log_{10} CFU/mL).

Table 2. Comparison of the average aerobic mesophilic, *Enterobacteriaceae*, and psychrotrophic counts across the sample groups.

	Log$_{10}$ colony-forming units (CFU)/mL		
Sample group	Mesophiles	*Enterobacteriaceae*	Psychrotrophs
A	7.48 b	5.8 b	2.18 b
B	9.12 a	8.91 a	5.62 a
C	8.27 ab	7.63 ab	5.55 a
D	9.66 a	8.52 a	4.29 ab
Mean	8.63	7.71	4.41
Coefficient of variation (%)	15.34	62.02	28.75

Means with the same letter are not significantly different ($p > 0.05$, analysis of variance followed by the Tukey test).

Statistically, producers B and D presented the highest bacterial loads, indicating the poorest hygiene practices. These levels dramatically exceed the Brazilian legal limit for raw milk (3×10^5 CFU/mL or 5.48 log$_{10}$ CFU/mL) [21] with nearly all samples surpassing this threshold. This widespread contamination suggests significant hygienic shortcomings, likely stemming from diverse sources. While the mesophilic count serves as a general indicator with differing legal limits worldwide (e.g., the Unites States, Europe, New Zealand, and Canada) [37], the high counts observed in this study surpass those reported in some earlier Brazilian investigations [26,38] and are comparable to bacterial levels found in cattle manure, thereby presenting considerable public health risks [39].

3.2.2 *Enterobacteriaceae*

The alarmingly high *Enterobacteriaceae* counts in the raw milk samples (Table 2)—with an overall average of 7.71 log$_{10}$ CFU/mL and a concerning peak of 11.27 log$_{10}$ CFU/mL—highlight critically poor hygiene practices during production, storage, and packaging. Statistical analyses revealed a pattern consistent with the mesophilic counts, as sample groups B and D also exhibited the highest levels of *Enterobacteriaceae*. These elevated levels, anticipated given the lack of hygienic and sanitary control during production, storage, and packaging, strongly suggest fecal and non-fecal contamination, posing a significant public health risk due to the potential for pathogen transmission. These values are considerably higher than those reported in previous studies: Pyz-Łukasik *et al.* [40] reported a range of 4.67 to 4.94 log$_{10}$ CFU/mL, Sobeih *et al.* [41] noted an average of 6.00 ± 5.29 log$_{10}$ CFU/mL, and El-Mokadem *et al.* [42] reported an average of 5.02 and 5.62 log$_{10}$ CFU/mL for raw milk from government and private farms, respectively. Consistent with the conclusions of those studies, which deemed milk unsafe due to high *Enterobacteriaceae* counts, our findings of even greater contamination levels lead us to the same conclusion.

3.2.3 Psychrotrophs

The psychrotrophic counts (Table 2) varied significantly among the four producers. Producer A showed the lowest mean count (2.18 log$_{10}$ CFU/mL), meanwhile producers B (5.62 log$_{10}$ CFU/mL) and C (5.55 log$_{10}$ CFU/mL) had the highest averages. This suggests their milk was more likely to have poorer sensory attributes because these microorganisms generate lipases and proteases, enzymes that break down milk components over time, causing spoilage, reduced yield, and changes in taste and texture [43]. Such contamination typically arises from inadequate hygiene, poor water quality in the milking environment, and contaminated equipment [37]. Although Brazilian legislation does not specify limits for psychrotrophic counts, a threshold of 5.11 log_{10} CFU/mL is deemed adequate to impair milk quality [43]. Over half (52.5%) of the samples in this study exceeded this value.

3.2.4 Total and thermotolerant coliforms

The average total coliform count was 4.15 log_{10} most probable number (MPN)/mL, specifically 1.17-4.38 log_{10} MPN/mL for producer A, 1.30-5.04 log_{10} MPN/mL for producer B, 2.46-5.04 log_{10} MPN/mL for producer C, and 2.55-5.04 log10 MPN/mL for producer D. The average thermotolerant coliform count was 3.34 log_{10} MPN/mL (**A**: 1.17-3.04 log_{10} MPN/mL; **B**: 1.17-3.04 log_{10} MPN/mL; **C**: 0.47-4.38 log_{10} MPN/mL; **D**: 2.32-3.96 log_{10} MPN/mL). A high thermotolerant coliform count, which is an established indicator of poor food hygiene, strongly suggests inadequate milking practices. Contamination likely originated from various sources, like the environment, animals, and equipment [40,44].

Although Brazilian law does not require coliform analysis of raw milk, the observed levels significantly surpass the 100-1000 MPN/mL threshold for poor hygiene [37], indicating likely fecal contamination. This is further supported by the detection of *Escherichia coli*, a key fecal indicator, in 27.5% of the samples, with average count of 3.57 log_{10} CFU/mL. This presence was expected given the high mesophilic and other coliform counts [42]. The potential for *E. coli* to spread antibiotic-resistance genes highlights its public health significance within the One Health perspective [44,45].

3.2.5 *Salmonella* spp.

Despite the high overall microbial contamination, we did not detect *Salmonella* spp. in any of the raw milk samples. This absence might be because Salmonella has a weaker ability to compete in such heavily contaminated environments. However, in a review of Brazilian milk quality studies, Müller and Rempel [22] revealed a concerning picture. Their analyses shows that informal milk not only has high counts of various bacteria but also contains *Salmonella* spp., *Staphylococcus* spp., fungi, and antimicrobial residues, highlighting its generally low quality.

3.3 Disinfectant resistance

The average inhibition zone (Table 3) and statistical evaluation (Table 4) revealed comparable disinfectant effectiveness against mesophilic bacteria, *Enterobacteriaceae*, and total coliforms.

Table 3. Average inhibition zones formed by the tested disinfectants against microorganisms isolated from the raw milk samples.

	Average inhibition zone (mm)		
Disinfectant	Mesophiles	*Enterobacteriaceae*	Total coliforms
A	0	0	0.6
B	10.91	12.33	11.71
C	9.85	9.42	8.38
D	18.15	18.76	18.9
E	1.83	1.47	1.72

Table 4. Comparison between the average inhibition zones of the tested disinfectants.

Disinfectant	Average inhibition zone (mm)	
D	18.15	a
B	10.91	b
C	9.85	b
E	1.83	c
A	0	c

Means with the same letter are not significantly different ($p > 0.05$, one-way analysis of variance followed by the Tukey test).

Disinfectants D, B, and C demonstrated antimicrobial action, with D displaying the highest potency. There was no significant difference between the effects of B and C. In contrast, disinfectants A and E did not exhibit any sanitizing capability. Although Rozman *et al.* [(46)] pointed to scarce research and possible resistance to certain disinfectants, such as H_2O_2, we found that H_2O_2 produced an average inhibition zone ranging from 10.91 to 12.33 mm, suggesting susceptibility despite the absence of established reference values.

Notably, 2.5% sodium hypochlorite proved to be the most potent sanitizer, producing the largest inhibition zones (with an average of 18.15-18.90 mm), which aligns with the findings reported by Melo and Coelho [(47)] against different pathogenic organisms. However, the observation by Negreiros *et al.* [(48)] of Enterococcus faecalis proliferation following exposure to this concentration raises concerns about the increasing bacterial resistance to this commonly used disinfectant and emphasizes the need to assess its efficacy across a broad range of microbial environments.

The intricate evolution of resistance to disinfectants, involving mechanisms such as genetic mutation [(46)] in the case of quaternary ammonium compounds, and the diminished efficacy of sodium hypochlorite in environments rich in organic matter, which are typical in milk production [(48)], further complicates strategies for disinfection. Furthermore, residual levels of disinfectants can favor the survival of tolerant microorganisms. Considering the limited availability of comparative data, the possibility of resistance or reduced sensitivity to disinfectants employed in milk production is particularly worrisome. The tested microorganisms are crucial indicators of hygiene and sanitation, underscoring the vital importance of ongoing research to guarantee effective disinfection protocols.

4. Conclusion

We showed that raw milk informally commercialized online in the municipality of Cuiabá is of low microbiological quality and is frequently adulterated. This situation is fueled by a culturally ingrained but scientifically unfounded belief in its nutritional superiority over processed milk. This illegal online trade bypasses inspection, posing a clear public health risk that necessitates public awareness campaigns and active sanitary surveillance to curb both traditional and online sales. Furthermore, the observed bacterial resistance to common disinfectants underscores the critical need for proper disinfectant usage (i.e., the correct dosage and the use of good-quality water) and further research to develop solutions and preventative measures. Overall, the findings reveal failures in good hygiene practices, resulting in poor-quality final products that, combined with fraudulent activities, create a serious food safety risk.

Conflicts of interest statement

The authors declare that there is no conflict of interest.

Data availability statement

The data will be provided upon request to the corresponding author.

Author contributions

Conceptualization: C. M. Decol and E. S. de Almeida Filho. Data curation: G. B. Zaffalon, E. de P. Santos and C. M. Decol. Formal analysis: C. M. Decol. Investigation: G. B. Zaffalon and E. de P. Santos. Methodology: C. M. Decol, E. S. de Almeida Filho and G. B. Zaffalon. Project administration: G. B. Zaffalon. Resources: C. M. Decol, E. S. de Almeida Filho and G. B. Zaffalon. Supervision: C. M. Decol, E. S. de Almeida Filho. Visualization: G. B. Zaffalon. Writing – original draft: G. B. Zaffalon. Writing – review & editing: C. M. Decol, E. S. de Almeida Filho and G. B. Zaffalon.

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