Research Article

The Effect of Fermentation on the Physicochemical Quality and the Presence and Levels of Pesticide Residue in Cow Milk

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The study investigated the effect of spontaneous fermentation time (0, 12, and 24 h) on the physicochemical properties (pH, titratable acidity, total soluble solid, and colour) and the levels of pesticide residue in cow milk using standard analytical procedures. Expectedly, the pH and titratable acidity were inversely related, where the pH significantly ($p = 0.001$) decreased from 5.7 to 3.8 and titratable acidity significantly ($p = 0.001$) increased from 2.7 to 7.5% after the 24 h fermentation period. Like pH, the total soluble solid significantly declined from 10.7 to 3.8 °brix after the 24 h fermentation. Generally, fermentation resulted in significant ($p < 0.05$) changes in all the quantitative parameters evaluated. Pesticides were not detected in about 85% of the milk samples. Only chlorpyrifos was detected in about 15% of raw milk samples in concentrations above the Maximum Residue Limit (MRL) (0.0670). Interestingly, chlorpyrifos was not detected in milk samples after 24 h of spontaneous fermentation.

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1. Introduction

Milk and its products, such as fermented milk (yoghurt) and cheese, are well known for their protein, lipid, and other essential mineral qualities. Cow milk, the most substantially produced and consumed worldwide, contains a total solid content of 13%, 4% fat, 3.5% protein, and 5% lactose (Gidiglo, 2014).

Despite milk's numerous nutritional and health benefits to humans and animals, contamination with pesticide residues could lead to high health risks. Earlier work has shown that even in low

concentrations, the combined effect of persistent synthetic chemicals, such as pesticide ingestion, causes suppression of the immune response, hypersensitivity to chemical agents, breast cancers, reduced sperm count, and male sterility (Carvalho, 2006). There are reports of death cases as well as pesticide poisoning around the world, particularly in developing countries (Tariq et al., 2004). Infants are more at risk of adverse health effects as a result of pesticide contamination than adults after exposure because of their weak immune systems (Sajid, 2015).

Agrochemicals (fertilizers and pesticides) are an integral part of the current agricultural systems worldwide, and their use remains a common agricultural practice, particularly in the tropical world (Carvalho, 2006). Pesticides such as 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) and 1,2,3,4,5,6-hexa-chlorocyclohexane (HCH) are prohibited from use in developed nations because they are environmentally persistent. However, they are widely used in developing countries, including Ghana, because they are relatively cheaper, easy to synthesize, or they are given by developed nations (Fianko et al., 2011).

As an agriculture-based nation, pesticide use contributes much to Ghana's national development and public health programs. Since the inception of pesticides, their use to protect crops from pests has significantly reduced losses and improved the yield of crops such as cereals, vegetables, fruits, and other crops. Ghana thus has known a continuous growth of pesticide usage, both in the number of chemicals and quantities, because of the expansion of the area under cultivation for food, vegetables, and cash crops (MoFA, 2011).

Pesticides mostly used in Ghana include chlorpyrifos, dimethoate, diazinon, fenitrothion, Lambda-cyhalothrin, cypermethrin, and endosulfan. These pesticides are used to control foliar pests in crops such as pineapples, tomatoes, peppers, okra, eggplants, cabbages, and lettuces (Aboagye, 2002; Kyofa-Boamah & Blay, 2000). It is estimated that 87% of farmers in Ghana use chemical pesticides to control pests and diseases on vegetables and fruits (Dinham, 2003).

Several research studies conducted in Ghana have indicated the presence of organochlorine pesticide residues in fish, vegetables, water, sediments, breast milk, and blood samples (Ntow, 2001; Osafo-Acquaah, 1997).

Organochlorine pesticides are highly lipophilic in nature; therefore, their residues may easily concentrate in fatty foods such as milk products, leading to bioconcentration and biomagnification through the food chain (Darko & Acquaah, 2007).

Milk and its products (yoghurt, a local cottage cheese known as "wagashi," and a milk and millet beverage known as "Burkina") are sold and consumed throughout Ghana.

Several authors (Armenova et al., 2023; Petrova et al., 2022) have reported microbial detoxification of pesticides through fermentation. It has been shown that LAB of the Lactobacillus and Leuconostoc genera can metabolize a broad spectrum of synthetic insecticides and use them as their carbon and energy source through their esterase and phosphatase enzymes, as the mode of action (Markowiak & Śliżewska, 2017). Abou-Arab (Abou-Arab, 1997) also showed that DDT was degraded by 10.8–11.8% (1 ppm in milk and cheese) by the action of *Streptococcus thermophilus* and *L. bulgaricus. Leuc mesenteroides WCP907, L. plantarum WCP931, La. sakei WCP904, and Lev. brevis WCP902* isolates from kimchi were able to convert chlorpyrifos, with the *Lev. brevis WCP902* strain able to consume 83.3% of 30 mg/l of the pesticide in 3 days and completely assimilating it after 9 days (Cho et al., 2009).

This study, therefore, sought to investigate the effect of spontaneous fermentation on the presence and levels of pesticide residue in cow milk, as well as the physicochemical properties (pH, titratable acidity, total soluble solid, and colour) of the milk.

2. Methodology

2.1. Collection of Milk Samples

Thirteen fresh raw cow milk samples (1L) were randomly collected in major Fulani cattle farm settlements in the Northern region of Ghana. The samples were collected in sterilized airtight glass bottles and kept in a thermos-cool box containing ice, and immediately transported to the Spanish Laboratory UDS, Nyankpala campus in Tamale for spontaneous fermentation and physicochemical analysis, and then transported to the Ghana Standard Authority pesticide residue laboratory for pesticide analysis.

2.2. Milk Fermentation

About 500 ml of each of the raw milk samples were spontaneously (naturally) fermented in plastic containers for 24 h at normal room temperature.

2.2.1. pH and Titratable Acidity

The initial (0 h), mid (12 h), and final (24 h) pH of the fermented milk samples were determined using a Crison pH meter Basic 20. Acidity was determined by NaOH titration. Briefly, 10 mL of the milk sample was added to the phenolphthalein solution, and the mixture was titrated with NaOH 0.1 N until the color changed to pink for 30 s. Lactic acid was expressed as g lactic acid/L (Dimitrellou et al., 2019).

2.2.2. Total Soluble Solids (TSS)

The fermented samples were analysed for TSS using an MA871 Digital Sucrose Refractometer (HANNA instruments, Woonsocket, RI, USA). Before measuring the TSS content of the milk samples, the equipment was calibrated with distilled water. The TSS was then measured by placing a drop of the sample on the surface of the refractometer, and then the reading was taken on the screen. The TSS was measured in triplicates and expressed as ◦Brix.

2.2.3. Colour

The colour of the milk samples was determined using a handheld Chromameter (Model: Konica Minolta CR-410). In doing this, the Chromameter was first calibrated with a white tile, and each sample was poured to fill a petri dish and then covered. The lens of the Chromameter was placed on the petri dish at three different parts, and the colour was then taken. L* denotes darkness/lightness (0 = black, 100 = white), a* (- a = greenness and + a = redness), and b* (- b = blueness, + b = yellowness).

2.3. Pesticide Residues Analysis

The raw and fermented milk samples were placed in cool boxes containing ice packets and transported to the Ghana Standard Authority Pesticide Residue Laboratory (Accra) for analysis.

2.3.1. Pesticide Standards Preparation

Certified reference standards were obtained from LGC Standards with purity ranging between 98-99.99%. A stock solution of each standard was prepared by dissolving approximately 10 mg in 10 mL of ethyl acetate, and standards with purity below 99% were corrected for purity by adjusting the weight appropriately to obtain a stock of 1000 µg/mL.

2.3.2. Calibration and Fortification Standards

Mixed standard solutions of all the pesticides analysed were prepared by serial dilutions of the stocks in ethyl acetate. Different solutions were prepared for calibration and fortification purposes. Five (5) calibration levels of 0.005, 0.01, 0.02, 0.05, and 0.1 µg/mL were prepared for the organochlorines, while those for organophosphates and synthetic pyrethroids were 0.01, 0.02, 0.05, 0.1, and 0.2 µg/mL.

2.3.3. Sample Extraction

The extraction of the sample was based on the 'Quick, Easy, Cheap, Effective, Rugged and Safe' (QuEChERS) method. All laboratory glassware used for the analysis was thoroughly washed and further rinsed with acetone. Ten grams of the milk sample was extracted with 10 mL of acetonitrile in a centrifuge tube by vortexing for 2 min. Extraction salts (QuEChERS), made up of 4 g of magnesium sulphate ($MgSO_4$) anhydrous, 1 g of trisodium citrate dihydrate, 0.5 g of disodium hydrogen citrate sesquihydrate, and 1 g of sodium chloride, were then added and vortexed for 1 min. The samples were then centrifuged for 5 min at a speed of 3500 rpm to separate the solvents into layers. A spiked sample was also prepared with a known concentration for quality check.

2.2.4. Cleanup

The extract obtained (6 mL) was added to the cleanup salts (0.9 g $MgSO_4$ anhydrous, 0.15 g C18, 0.15 g PSA) in a 15 mL tube and vortexed for 30 seconds, and then centrifuged for 5 min at a speed of 3500 rpm. Four (4) mL of the cleaned extract was transferred into a 50 mL pear-shaped flask and acidified with 40 µL of 5% formic acid in acetonitrile. The extract was evaporated using a rotary evaporator to dryness; 1 mL of ethyl acetate was then added to reconstitute the extract with the aid of an ultrasonic bath. The final extract was then transferred into the Gas Chromatography (GC) vial containing 20 µL of 1% polyethene glycol in ethyl acetate.

2.3.5. Gas chromatography

A Varian CP-3800 gas chromatograph equipped with a 63Ni Electron Capture Detector (ECD) and Pulsed Flame Photometric Detector (PFPD) was used to determine the presence and levels of pesticide residues. The ECD, fitted with a fused silica capillary column, VF-5ms of 30 m length and 0.25 mm ID (internal diameter) along with 0.25 µm film thickness, was used to determine the presence and levels of organochlorines (OCs) and synthetic pyrethroids (SP) pesticides. Also, the PFPD, fitted with the

fused silica capillary, VF-1701P, 30 m length, 0.25 mm ID, and 0.25 µm film thickness, was used to determine the presence and levels of organophosphates (OPs) pesticides. Nitrogen gas of 99.999% purity was used as a carrier at a flow rate of 1 mL/min and make-up (29 mL/min) for the ECD. Nitrogen gas of 99.999% purity at a flow rate of 2 mL/min was the carrier gas, and hydrogen, Air1, and Air2 at 14, 17, and 10 mL/min, respectively, for the PFPD. The injector temperature was 250 °C; the ECD temperature was kept at 300 °C, and the PFPD at 280 °C. The column temperature program for the PFPD was at an initial temperature of 70 °C for 2 min, then increased to 200 °C at a rate of 25 °C/min and maintained for 1 min, and finally increased to 250 °C at a rate of 20 °C/min. For the ECD, the initial column temperature was 70 °C, kept for 2 min, then raised at a rate of 25 °C/min to 180 °C, kept for 1 min, and again raised at a rate of 5 °C/min to 300 °C. The injection volume was 1 and 2 µL for the ECD and PFPD, respectively.

2.3.6. Quality Control

To assure the validity of the results, reagent blanks and spiked samples were incorporated into the analytical batch. Blanks did not show any positive detects, and the recovery of all pesticides in spiked samples was in the range of 70 to 115 %. The limit of quantification (LOQ) was 0.005 mg/kg for organochlorines and 0.01 mg/kg for organophosphates and synthetic pyrethroids.

2.4. Data analysis

The data obtained were analyzed using Genstat Discovery Edition 4 Software 2013. Tables and graphs were used to represent the data graphically.

3. Results and Discussion

3.1. Physicochemical characteristics

Generally, the pH and titratable acidity (TA) respectively declined and increased as fermentation progressed up to 24 h (Figure 1). The significant decline in pH ($p = 0.001$) and the significant increase in TA ($p = 0.001$) with fermentation time are expected because the value of pH is inversely proportional to the lactic/organic acids content. The current findings lend support to earlier works by Akabanda et al. (2010) and Asefa et al. (2021), who reported similar trends in pH and TA with the fermentation of fresh cow milk. The fall in pH and increase in TA content of the fermented cow milk

could be ascribed to the buildup of some organic acids and acetic acid resulting from the actions of some fermentative organisms such as LAB and yeasts in the fermenting samples (Obadina et al., 2013). The reduction in pH and increased TA of the milk samples with fermentation is important in ensuring the safety of the samples.

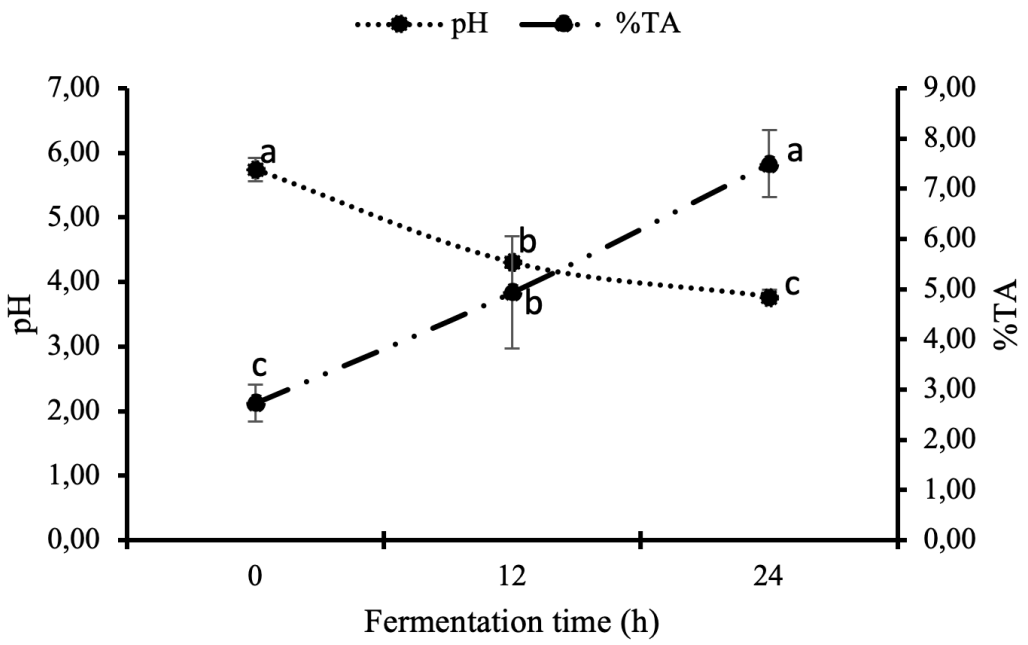

Figure 1. pH and titratable acids of fermented cow milk

Values are means ± SD. Means with different letters are significantly different ($p < 0.05$)

The total soluble solids (TSS) content denotes the quantity of total soluble solids in the milk sample. It is an important parameter because it does not only influence the rheology of the samples but also the taste, as it indicates the level of sweetness in the product. Thus, TSS mainly encompasses total sugar content and a fraction of soluble proteins, amino acids, and other organic materials (Bexiga et al., 2017; Setiawati et al., 2021). The TSS content in the current study significantly ($p < 0.001$) decreased with the progression of fermentation (Figure 2). This finding is in harmony with earlier works that showed a reduction in TSS in fermented milk (Setiawati et al., 2021). The decreased TSS with fermentation could be due to increased sugar metabolism and biotransformation, as reported by Adebo et al. (2021).

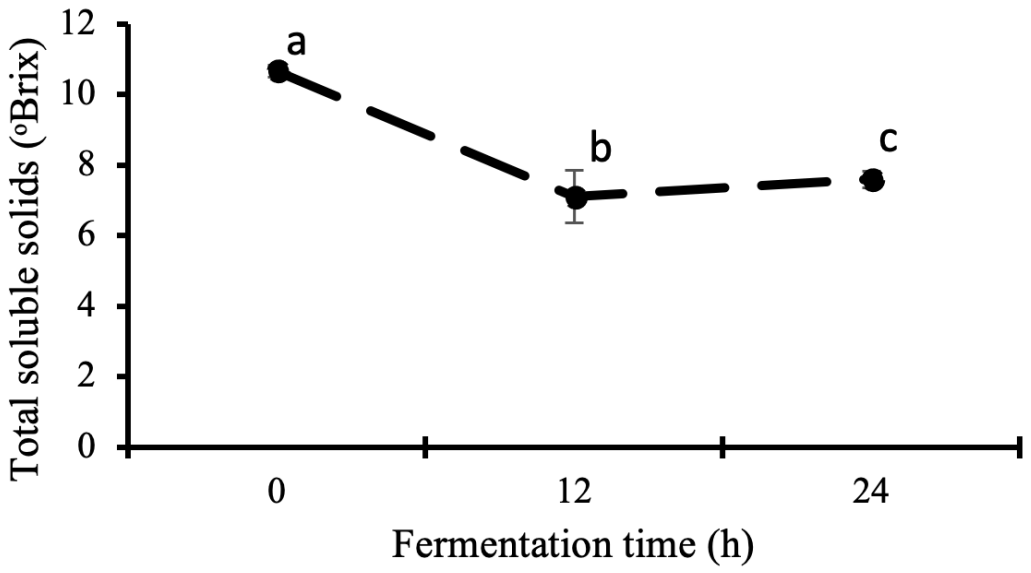

Figure 2. Total soluble solids of milk samples as influenced by fermentation time.

Values are means ± SD. Means with different letters are significantly different ($p < 0.05$)

Fermentation Time (h)	Colour parameters				
	L*	a*	b*	C	h
0	91.97 ±1.17^{a}	-5.31±0.25^{b}	11.67±1.8^{a}	12.84 ±1.65^{b}	114.82 ± 3.63^{a}
12	78.92±13.8^{b}	-4.24±1.21^{a}	16.99±7.0^{b}	17.70 ±6.55^{a}	107.04 ± 9.32^{b}
24	78.05 ±9.82^{b}	-3.98±1.63^{a}	17.25±9.4^{b}	18.00 ±8.92^{a}	107.72 ±10.71^{b}
p-value	0.001	0.001	0.023	0.029	0.003

Table 1. Effect of fermentation time on the instrumental colour parameters of milk samples.

The mean values of L* (luminosity), a* (redness), b* (yellowness), hue angle (h*), and chroma (C*) for the milk samples as influenced by fermentation time are presented in Table 1. The importance of food colour cannot be overemphasized because of its nexus with consumers' choice of a food product.

According to the American Meat Science Association (2012), visual appraisals of colour in foods are closely related to consumer or taster evaluations and set the benchmark for instrumental measurement comparison, such as those performed in the present study. Fermentation significantly ($p = 0.001$) reduced the luminosity (L*) and redness (a*) of the milk samples. However, the yellowness increased significantly ($p = 0.023$) with increased fermentation time. The present findings corroborate earlier work by Mkadem et al. (2023), who reported reduced luminosity with fermentation in cow milk. The consortium of microorganisms influenced the color profile of fermented milk. For example, organic compounds derived from lactic bacteria, such as reuterin produced by *Lb. reuteri*, could also influence the color of fermented milk. It was reported that fermented milk products without reuterin had higher L* values than fermented milk products with reuterin. However, fermented milk products with reuterin displayed higher a* and b* values (Ortiz-Rivera et al., 2017).

3.2. The presence and levels of pesticide residue

Results from the analysis of milk samples using the gas chromatograph showed no pesticide residue in about 85% (results not shown) of the raw and fermented milk samples (Tables 2 and 3). On the contrary, studies conducted by other authors (Gebremichael et al., 2013; Gill et al., 2020; Ishaq & Nawaz, 2018; Kampire et al., 2011) revealed the presence of pesticides in milk samples.

The only pesticide detected was chlorpyrifos (0.067 mg/kg) (Table 2) in raw milk samples (about 15% results not shown). The level of chlorpyrifos found in this study is far above the European Union maximum residue limit (EU MRL) of 0.01 mg/kg for milk. Chlorpyrifos [O, O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is an organophosphorus insecticide, acaricide, and nematicide that is used in controlling a broad range of foliage and soil-borne insect pests on a wide variety of foods and feed crops (Food et al., 1997; Lemus & Abdelghani, 2000). It is ranked as one of the most extensively used insecticides all around the world (Lu et al., 2014). This could explain its presence in the raw milk sample of this study.

Several studies have implicated chlorpyrifos in milk samples. Gill et al. (Gill et al., 2020) detected chlorpyrifos among other pesticides as the most common organophosphate in the milk of cattle. Chlorpyrifos was detected in milk samples that were above the MRLs, according to Kathpal et al. (Kathpal et al., 2001). Similarly, Cheema et al. (Cheema et al., 2004) found chlorpyrifos in 6.7% of milk samples from Punjab, India, with concentrations above MRL values. Recent studies have found that

OPs such as chlorpyrifos residues in milk are associated with their capacity to form covalent bonds with milk proteins (Bedi et al., 2005; Pagliuca et al., 2006).

The absence of other pesticide residues may be explained by the high volatility of these chemical residues, and also, the grazing pasture drying up during the dry season when samples were collected, leaving hardly any residues. According to Lu et al. (2014) and Fantke et al. (2014), plant features and environmental factors, including temperature, light, moisture, and pH, have an impact on pesticide dissipation. Additionally, pesticide residues in the feed may have decayed or fallen below the detection threshold, the contaminated feed component may no longer be present on the farm, and it might have run off into nearby water bodies from the grazing areas. The absence of pesticide residues in our samples may be owing to the difficulty in detecting pesticide residues in water bodies due to their dilution effects, hydrolysis, and photolysis on superficial waters (Fantke et al., 2014).

It is possible that microbial degradation could be responsible for the absence of pesticide residue in the fermented milk samples (Aislabie & Lloyd-Jones, 1995). One of the most significant mechanisms for the breakdown of many organic compounds is the biodegradation of chemicals by microbes, which could also account for the absence during fermentation (Bayarri et al., 1997; Sarmah et al., 2009). Many different types of chemical pesticides can be broken down by bacteria's extracellular enzymes. These microbes obtain their carbon and energy from the pesticide’s residues. Numerous types of research have examined both the qualitative and quantitative facets of the breakdown of pesticides as a result of the influence of microorganisms. A study by Maden and Kumral (Maden & Yildirim Kumral, 2020) investigated the degradation of various insecticides in sauerkraut samples with or without the presence of LAB during fermentation. Lactobacillus plantarum (109 cfu/ml) contributed to malathion (2 mg/kg) and chlorpyrifos-methyl (4 mg/kg) degradation. They again tested Lab. plantarum strains for pesticide removal in the course of black olive fermentation, and at the end of fermentation (after 60 days), 61% of deltamethrin, 68% of dimethoate, and 50% of imidacloprid were removed. Lactobacillus pentosus was applied to successfully eliminate beta-cypermethrin from silage, degrading about 96% of the pesticide with a concentration of 50 mg/L (Liu et al., 2022).

Pesticide residues on agricultural outputs and in food are contaminants that endanger the lives of consumers. Therefore, the presence of pesticide residues in food is deemed hazardous to human health (Kovacova et al., 2014; Lozowicka, 2015). The production of DNA and the number of cells in some parts of the human brain may be harmed by exposure to chlorpyrifos, even at low concentrations, during pregnancy or shortly after delivery, according to toxicology studies (Campbell

et al., 1997; Dam et al., 1998; Whitney et al., 1995). Low-level exposure to chlorpyrifos may harm the neurological system's function later in life, which may have repercussions for normal learning and behaviour (Eskenazi et al., 1999). Children may be more susceptible to chlorpyrifos than adults, according to studies done on animals, as a fetus's or a young animal's neural system may be more than 20 times more sensitive to the pesticide than an adult's neurological system (Moser et al., 2015).

Pesticide		Results (mg/kg)	EU MRLs (mg/kg)
Aldrin		Not detected	0.006[a]
Allethrin		Not detected	0.01[d]
Alpha-Endosulfan		Not detected	0.05[b]
Beta-Endosulfan		Not detected	0.05[b]
Beta-HCH		Not detected	0.01
Bifenthrin		Not detected	0.02
Chlorfenvinphos		Not detected	0.01
Chlorpyrifos		**0.067**	0.01
Cyfluthrin		Not detected	0.02
Cypermethrin		Not detected	0.05
Delta-HCH		Not detected	0.01[d]
Deltamethrin		Not detected	0.05
Diazinon		Not detected	0.02
Dieldrin		Not detected	0.006[a]
Dimethoate		Not detected	0.01
Endosulfan Sulfate		Not detected	0.05[b]
Ethoprophos		Not detected	0.01
Fenitrothion		Not detected	0.01
Fenpropathrin		Not detected	0.01[d]
Fenvalerate		Not detected	0.01[d]
Fonofos		Not detected	0.01[d]
Lambda-cyhalothrin		Not detected	0.02
Lindane		Not detected	0.01

Pesticide		Results (mg/kg)	EU MRLs (mg/kg)
Malathion		Not detected	0.02
Methamidophos		Not detected	0.01
Methoxychlor		Not detected	0.01
p, p'-DDD		Not detected	0.04[c]
p, p'-DDE		Not detected	0.04[c]
p, p'-DDT		Not detected	0.04[c]
Parathion		Not detected	0.05
Permethrin		Not detected	0.01[d]
Pirimiphos-methyl		Not detected	0.01
Profenofos		Not detected	0.01

Table 2. Pesticides tested in Raw milk samples

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Pesticide	Results (mg/kg)	EU MRLs (mg/kg)
Aldrin	Not detected	0.006[a]
Allethrin	Not detected	0.01[d]
Alpha-Endosulfan	Not detected	0.05[b]
Beta-Endosulfan	Not detected	0.05[b]
Beta-HCH	Not detected	0.01
Bifenthrin	Not detected	0.02
Chlorfenvinphos	Not detected	0.01
Chlorpyrifos	Not detected	0.01
Cyfluthrin	Not detected	0.02
Cypermethrin	Not detected	0.05
Delta-HCH	Not detected	0.01[d]
Deltamethrin	Not detected	0.05
Diazinon	Not detected	0.02
Dieldrin	Not detected	0.006[a]
Dimethoate	Not detected	0.01
Endosulfan Sulfate	Not detected	0.05[b]
Ethoprophos	Not detected	0.01
Fenitrothion	Not detected	0.01
Fenpropathrin	Not detected	0.01[d]
Fenvalerate	Not detected	0.01[d]
Fonofos	Not detected	0.01[d]
Lambda-cyhalothrin	Not detected	0.02
Lindane	Not detected	0.01
Malathion	Not detected	0.02

Pesticide	Results (mg/kg)	EU MRLs (mg/kg)
Methamidophos	Not detected	0.01
Methoxychlor	Not detected	0.01
p, p'-DDD	Not detected	0.04[c]
p, p'-DDE	Not detected	0.04[c]
p, p'-DDT	Not detected	0.04[c]
Parathion	Not detected	0.05
Permethrin	Not detected	0.01[d]
Pirimiphos-methyl	Not detected	0.01
Profenofos	Not detected	0.01

Table 3. Pesticides tested in fermented milk samples

4. Conclusion

The present study investigated the effect of fermentation time (0, 12, and 24 h) on the physicochemical properties (pH, titratable acidity, total soluble solid, and colour) and the levels of pesticide residue in cow milk using standard analytical procedures. Except for Chlorpyrifos, no pesticide residue was detected in the raw milk sampled. However, after 24 h of spontaneous fermentation, no pesticide residue was detected. The study revealed that the Chlorpyrifos concentration detected was above its EU MRLs for milk, and this may be dangerous for human consumption, especially children.

Statements and Declarations

Competing Interest

The authors declare that they have no known competing financial or personal interests that could have appeared to influence the work reported in this paper.

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Authors' contribution

FA designed the work, analyzed, and interpreted the data; EA interpreted the data and critically revised the manuscript; RAA participated in the conception of the work and analyzed and interpreted the data. All authors read and approved the final version of the manuscript.

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