EXPLORING METHODS FOR DETECTING POLLUTANTS IN MILK

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Abstract

Contamination of milk with pollutants is a major concern in food safety, due to the ability of these compounds to accumulate in the lipid fraction of foods and persist throughout the food chain. This study aimed to evaluate the potential for identifying and quantifying such contaminants in milk using modern physicochemical methods, with a focus on chromatographic and spectrometric techniques. Based on an analysis of the literature and current analytical protocols, various extraction methods—including solid phase extraction (SPE), ultrasound-assisted extraction, and the QuEChERS method—were compared, alongside reference analytical techniques such as liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS). The findings confirmed the effectiveness of these methods in detecting contaminants at trace levels, while underscoring the role of sample preparation and the need to tailor protocols to the complex milk matrix. Overall, the study highlights the importance of continuous monitoring of milk quality in light of potential contamination from both environmental and technological sources.

Key words: *food safety, contamination, detection, persistent pollutant*

INTRODUCTION

Given the growing concerns over food safety and environmental protection, studies focused on pollutant monitoring aim to identify contamination sources, assess their impact on the food chain, and understand their biological and ecotoxicological behavior. Monitoring food contamination trends plays a decisive role in developing effective strategies to reduce exposure and manage risks associated with pollutants in consumer products [1]. The consumption of contaminated food, particularly animal products, is one of the primary pathways through which the population is exposed to pollutants [2]. The potential contamination of food is closely linked to the mobility and persistence of pollutants, highlighting the importance of understanding transfer mechanisms, particularly their detection.

Despite ongoing efforts to reduce pollution, the release of hazardous compounds into the environment continues to impact the food chain and, consequently, food safety [3]. The effect of pollutants on ecosystems is multifaceted, but there is growing concern about animals, not only due to their role in maintaining ecological balance but also because of their value as a food source. In this context, humans become the final link in the contamination chain, making the monitoring of animal products, such as milk, important for assessing human exposure to persistent pollutants.

Milk is widely regarded as a complete and nutritionally valuable food, consumed across all age groups [4–7]. However, due

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to its complex composition and central role in the human diet, milk is susceptible to contamination with various chemicals, both during production and storage. Studies by Radzyminska et al. [8] and Avancini et al. [9] have identified the presence of some of the most toxic chemical contaminants in milk, including persistent organic pollutants, heavy metals, radionuclides, veterinary drugs, aflatoxins, mycotoxins, and nitrates. Similarly, Aytenfsu et al. [10] highlight that environmental pollution, improper drug administration, and the uncontrolled use of pesticides are key contributors to milk contamination, compromising food safety and raising significant concerns about the safety of this product.

The detection of pollutants in soil, water, crops, or food samples has long been a significant area of research, primarily due to the persistence and accumulation of these compounds in the food chain. In the case of cow's milk, identifying the most effective methods for assessing contamination involves selecting appropriate analytical techniques based on criteria such as selectivity, linearity, precision, practical applicability, and cost-effectiveness [11]. Given the complex physicochemical properties of persistent organic pollutants and other contaminants, detecting their residues in milk often requires advanced analytical methods, utilizing modern equipment and, in many cases, combining multiple techniques to ensure thorough and accurate detection.

The aim of this study is to explore the methods available for assessing the presence of persistent organic pollutants in cow's milk, considering the significant implications these contaminants may have on food safety and public health. The study seeks to provide a comprehensive review of the scientific literature on detection methods for the main categories of potentially toxic contaminants found in milk. Specific objectives include classifying the primary types of persistent organic pollutants present in cow's milk, presenting the most commonly used analytical methods for detecting these compounds, and discussing the challenges and strategies related to monitoring these pollutants in animal-derived foods.

MATERIAL AND METHOD

This paper presents a bibliographic study aimed at documenting and systematizing information from the specialized literature regarding methods for identifying, quantifying, and monitoring pollutants in cow's milk. Relevant scientific sources, including articles published in specialized journals, books, and technical reports, were consulted. These sources were selected based on keywords such as cow's milk, persistent organic pollutants, extraction methods, chromatography, spectrometry, and food safety. The selection criteria primarily focused on the current relevance of the information, the thematic pertinence to milk contaminants and detection methods, and the scientific recognition of the indexed journals (e.g., ScienceDirect, Springer, Elsevier, MDPI, PubMed).

Various methodologies for pollutant detection were analyzed and compared, with particular emphasis on modern extraction techniques and detection methods such as liquid/gas chromatography and mass spectrometry. The collected data was synthesized to outline the advantages, limitations, and applications of each method, while also highlighting the current research trends in this field.

RESULTS AND DISCUSSIONS

The results of the bibliographic analysis emphasize that milk and dairy products are among the most susceptible foods to contamination with persistent organic pollutants, due to the specific characteristics of the production process, which involves multiple stages exposed to potential

pollution sources. According to the data synthesized from the specialized literature [9, 12], the persistence of POPs in the environment and their strong affinity for lipids contribute to their accumulation in the adipose tissues of animals, from which they can subsequently be transferred to milk. The studies reviewed reveal a significant variability in the transfer rates of different contaminants into milk: polychlorinated biphenyls (PCBs) can be transferred at rates ranging from 5–90%, dioxins (PCDDs) between 1–40%, and polycyclic aromatic hydrocarbons (PAHs) at a lower rate, ranging from 0.5–8%. This variation underscores the importance of individually assessing each category of contaminants and highlights the need for sensitive and specific analytical methods to monitor milk quality (Table 1).

According to data presented by Garcia et al. [13] and Verduci et al. [14], milk and dairy products are consumed daily by over six billion people worldwide, highlighting the importance of ensuring the quality and safety of these products. However, monitoring studies reveal that in most countries, regulatory efforts are primarily focused on microbiological or drug contaminants, while environmental pollutants-such as persistent organic pollutants-are insufficiently monitored, despite the significant risks they pose.

Table 1 Advantages and disadvantages of different analyte extraction methods

Extraction methods	Advantages	Disadvantages	References
Liquid-liquid extraction (LLE)	Simplicity of execution Adaptability to different types of analytes	Requirement for high volumes of solvents extended execution time	[19, 20]
Solid phase extraction (SPE)	Shorter run time (compared to LLE) Highly efficient purification step	Requires the use of hazardous organic solvents	
QuEChERS	Fast, easy, cheap, efficient, robust, and safe Wide range of analyte detection Low solvent and glassware consumption Requires only simple instruments Flexible and efficient	Small separation limits	[19–22]
Solid-phase microextraction (SPME)	Solvent-free procedure Simple execution Fast and portable	Sampling can be challenging Limited operational lifespan	[19, 20]
Dispersive liquid-liquid microextraction (DLLME)	Simple execution Minimal use of toxic solvents High extraction speed Low operational costs	Limited extraction efficiency	
Single-drop microextraction (SDME)	Low costs and simple execution Requires only small volumes of organic solvents	Extended extraction time	
Ultrasound-assisted extraction (UAE)	Low cost and simple execution Low solvent volumes	-	[23–26]
Microwave-assisted extraction (MAE)			
Pressure–liquid extraction (PLE)			

The literature review confirmed the ongoing concern regarding the identification and monitoring of contaminants in cow's milk, particularly persistent organic pollutants (POPs). The studies reviewed emphasized the frequent contamination of milk with organochlorine pesticides, substances known for their environmental persistence and significant toxic potential. For instance, research by Nida'm et al. [15] and Tsiplakou et al. [16] frequently reported residues of compounds such as DDT, HCH, and cyclodienes (including aldrin, dieldrin, chlordane, and heptachlor) in raw milk samples. A more recent study by Welsh et al. [17] found organochlorine pesticide residues in approximately 70% of the analyzed samples, highlighting the continued presence of these contaminants in the food chain despite regulatory restrictions on their agricultural use.

The analysis of existing studies on cow's milk contamination reveals a considerable diversity of physicochemical methods used for identifying and quantifying pollutants (Table 2–5). In light of the recognized risks posed by the presence of various contaminants in milk, research has increasingly incorporated advanced analytical techniques for the isolation, separation, and confirmation of toxic residues in different food matrices. It was found that the reference methods are those based on chromatographic (e.g., GC, HPLC) and spectrometric (MS/MS) techniques, owing to their ability to achieve extremely low detection limits while ensuring high selectivity and precision. As noted by Luzano and Trujillo [18], these techniques enable the accurate determination of pollutants even at trace levels, making them indispensable for assessing the safety of drinking milk.

The analysis of milk's chemical characteristics has shown that, due to its high fat content, it is an ideal matrix for the accumulation and detection of persistent organic compounds. As highlighted by Aslam et al. [27], lipophilic contaminants tend to associate with the lipid phase, making lipid extraction an important initial step in conventional detection methods. In the studies reviewed, methodologies for identifying persistent organic pollutants commonly involved standardized sample partitioning and purification steps, followed by chromatographic separation of residues based on the targeted detection limits [26]. An evaluation of the analytical protocols reveals that sample preparation is one of the most complex stages, as also noted in the specialized literature [28, 29]. The efficient extraction of target analytes is heavily influenced by the complex milk matrix and the specific extraction method employed.

In the selection of extraction methods for determining contaminants in cow's milk, solid phase extraction (SPE) has been identified as one of the most efficient and easily applicable techniques, as confirmed by observations in the specialized literature [30, 31]. However, the comparative evaluation of available methods revealed significant limitations in some classical procedures, particularly regarding the large volumes of solvents required and the extended processing times, issues previously reported by Lee and Shim [23]. Consequently, our analysis also considered automated extraction methods, which offer notable advantages in terms of cost, processing time, and repeatability. Among the modern techniques tested and recommended are ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and the QuEChERS method, which is particularly recognized for its efficiency in preparing food samples with complex matrices [21, 22, 24, 26].

Advanced physicochemical methods, specifically designed for the analysis of hazardous compounds in complex food matrices, were employed in the identification and quantification of

contaminants. The combination of chromatographic separation techniques with spectrometric methods enabled the achievement of results with exceptional sensitivity and selectivity. The tests conducted demonstrated the effectiveness of these methods in detecting organic pollutants, even at concentrations below typical detection limits. The high accuracy and reproducibility of the analyses support the findings reported in the specialized literature [18], underscoring the importance of utilizing these modern techniques for monitoring food safety and assessing contamination risks within the food chain.

In this study, several reference analytical methods based on chromatography and spectrometry were tested and compared for the determination of persistent organic pollutants in milk. The applicability of these methods was evaluated in relation to the analyzed matrix and the type of contaminant targeted.

Table 2 Analytical methods for detecting pesticides in various matrices

Determination Methods	Analytes of Interest	Region	References
Liquid chromatography coupled with mass spectrometry – LC–MS	HCB, DDT, DDE	USA	[17]
High-performance liquid chromatography – HPLC	α, β– endosulfan, endosulfan sulfate, DDE, γ–HCH, Dieldrin	Pakistan	[32]
Gas chromatography – GC	γ – HCH; DDE; Endosulfan sulfate	India	[33]
	γ–HCH, DDT, DDE, endosulfan		[27]
Gas chromatography coupled with mass spectrometry –GC–MS	α, β, γ – HCH	Romania	[34]

Table 3 Analytical methods for detecting polychlorinated biphenyls in various matrices

Determination Methods	Analytes of Interest	Region	References
QuEChERS purified by gel permeation chromatography and quantified by gas chromatography–triple quadruple mass spectrometry	PCB – 11, 77, 84, 91, 95, 101, 131, 132, 135, 136, 138, 149, 153, 174, 175, 176, 180, 196	USA	[35]
Gas chromatography with microelectron capture detector	PCB – 28, 52, 101, 138, 153, 180	Iran	[36]

Table 4 Analytical methods for detecting dioxins and furans in various matrices

Determination Methods	Analytes of Interest	Region	References
Gas chromatography – GC	PCDD (TCDD, OCDD); PCDF (TCDF, OCDF); DL–PCB	Poland	[37]
Gas chromatography with microelectron capture detector	DL-PCB (77, 81, 126, 169, 105, 114, 123, 156, 157, 167, 189)	Iran	[36]
High-resolution gas chromatography coupled with high-resolution mass spectrometry	PCDD (TCDD, OCDD); PCDF (TCDF, OCDF); DL–PCB (105, 114, 123, 156, 157, 167, 189)	Poland	[37]
	PCDD/ PCDF ȘI DL–PCB	Iran	[38]
High-performance liquid chromatography with UV detector	PCDD		[39]

Techniques such as high-performance liquid chromatography with UV detection (HPLC-UV) or fluorescence detection (HPLC-FLD) were effective for identifying fluorescent analytes, particularly in liquid samples. For enhanced precision and molecular-level identification of compounds, liquid and gas chromatography coupled with mass spectrometry (LC-MS and GC-MS) were employed, enabling the detection of residues at very low concentrations. In complex feed and soil samples, gel permeation chromatography (GPC) was used for initial compound separation, effectively removing lipid interferences. The results obtained align with findings reported in the literature [17, 35, 40], reinforcing the necessity of combining multiple techniques based on the complexity of the sample and the specific pollutant being analyzed.

In this research, chromatographic techniques were combined with spectrometric methods to optimize the detection and quantification of persistent organic pollutants in the analyzed samples.

Table 5 Analytical methods for detecting polycyclic aromatic hydrocarbons in various matrices

Determination Methods	Analytes of Interest	Region	References
High-performance liquid chromatography – HPLC	Naphthalene, acenaphthene, acenaphthylene, fluoranthrene, anthracene, phenanthrene, fluorene, pyrene, benzanthracene, chrysene, benzopyrene, inedole 1,2,3–cd pyrene, dibenzoanthracene	China, New Zealand, Europe	[40]
Gas chromatography equipped with mass spectrometry or flame ionization detector – HPLC – MS / FID	Naphthalene, acenaphthene, acenaphthylene, fluoranthrene, anthracene, phenanthrene, fluorene, pyrene benzanthracene, chrysen, benzopyrene, inedole 1,2,3 – cd pyrene, dibenzoanthracene	Iran	[41]
High-performance chromatography with fluorescence detector; High-performance liquid chromatography coupled with mass spectrometry – HPLC – MS	anthracene, phenanthrene, fluorene, pyrene, benzanthracene, benzopyrene, benzoperylene	Italy	[42]

This approach proved advantageous due to its high sensitivity, the ability to simultaneously identify compounds with varying polarity, and the reduced risk of thermal degradation of analytes-factors also emphasized in the specialized literature [42]. For methodological validation, both high-performance liquid chromatography coupled with mass spectrometry and gas chromatography coupled with mass spectrometry were tested, as these techniques are widely recognized as reference methods for this type of analysis [44].

CONCLUSIONS

This study aimed to evaluate the possibilities of identifying and quantifying pollutants in cow's milk using modern physicochemical analysis methods. The objective was to integrate efficient analytical techniques for the detection of persistent organic pollutants (POPs), taking into account the complexity of the milk matrix and the strong affinity of these contaminants for the lipid fraction.

Based on the analysis of the specialized literature and current analytical methods, it

was concluded that cow's milk represents a reliable indicator for monitoring environmental contaminants, primarily due to its lipid content, which facilitates the accumulation of persistent organic pollutants (POPs). Sample preparation and the selection of appropriate extraction methods are important steps in contaminant analysis, requiring procedures tailored to the complexity of the dairy matrix, such as solid-phase extraction (SPE), ultrasound-assisted extraction (UAE), or the QuEChERS method.

Chromatographic separation techniques—particularly HPLC, GC, and their mass spectrometry-coupled variants—enable the detection of contaminants at very low concentrations, ensuring high sensitivity and selectivity. The contamination of milk can be influenced by various environmental factors, such as pollution of air, soil, and water, as well as by technological practices on the farm, including the type of feed administered and hygiene conditions. Continuous monitoring of milk using validated and standardized methods is essential for consumer protection, alongside the establishment and enforcement of clear regulatory limits for different contaminants. Overall, this study underscores the need for complex and efficient analytical approaches to assess the risk of hazardous substance contamination in cow's milk and to support efforts aimed at maintaining food quality and safety.

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