DIET FACTOR
Journal of Nutritional & Food Sciences
https://www.dietfactor.com.pk/index.php/df
ISSN (E): 2789-8105, (P): 2789-8091
Volume 6, Issue 1 (Jan-Mar 2025)

**Original Article**

OPEN ACCESS

# Melamine a Potent Carcinogen: Detection in Packed and Unpacked Milk Samples Collected from Different Regions of Lahore, Pakistan by ELISA Method

**Naseem Zahra[1], Muhammad Khalid Saeed[1*], Areeba Javaid[2], Muntaha Shahid[2], Shamakh Naveed[2] and Asma Saeed[1]**

[1]Food and Biotechnology Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Pakistan
[2]Department of Microbiology, University of Agriculture, Faisalabad, Pakistan

## ARTICLE INFO

***Keywords:***
Melamine, Milk Safety, Packaged Milk, Food Contamination, Enzyme-Linked Immunosorbent Assay

***How to Cite:***
Zahra, N., Saeed, M. K., Javaid, A., Shahid, M., Naveed, S., & Saeed, A. (2025). Melamine a Potent Carcinogen: Detection in Packed and Unpacked Milk Samples Collected from Different Regions of Lahore, Pakistan by ELISA Method: Melamine Detection in Milk. DIET FACTOR (Journal of Nutritional and Food Sciences), 6(1), 19-24. https://doi.org/10.54393/df.v6i1.163

***Corresponding Author:**
Muhammad Khalid Saeed
Food and Biotechnology, Research Centre, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Pakistan
rosefbrc1@gmail.com

Received date: 27th January, 2025
Revised date: 2nd March, 2025
Acceptance date: 17th March, 2025
Published date: 31st March, 2025

## ABSTRACT

Melamine is a nitrogen-rich compound often added to milk to enhance its protein contents. The European Commission has established a permissible limit of 2.5mg/kg for melamine, as excessive amounts can lead to kidney stones and other health risks for both infants and adults. **Objective:** To assess melamine concentrations in both packed and unpacked milk samples from various regions in Lahore, Pakistan. **Methods:** An experimental study was conducted using the Enzyme-Linked Immunosorbent Assay (ELISA) technique to detect melamine levels in milk samples. Measurements were obtained at a wavelength of 450 nm, following standard ELISA protocols. **Results:** The analysis revealed that one unpackaged milk sample contained melamine levels exceeding the permissible limit at 6.3+0.1mg/kg, while the other samples were deemed safe for consumption. Additionally, while melamine was detected in packed milk samples, all were within safe consumption levels. **Conclusions:** In conclusion, melamine was not found in the packaged milk samples, adhering to the acceptable limits set by the European Commission in 2009; however, one unpackaged milk sample showed a concerning level of melamine.

## INTRODUCTION

Milk is very nutritious for people and serves as an energy source, supplying lactose, fats, proteins, calcium, vitamins, and minerals [1]. Adulteration refers to the deliberate addition, replacement with lower-quality stuff, or elimination of precious elements from food for personal gain, whereas food fraud encompasses the purposeful inclusion of harmful ingredients [2]. This study presented a colorimetric method for detecting melamine in milk using gold nanoparticle aggregation, offering a rapid and visual detection approach [3]. In most regions of Pakistan, low-quality milk is available, and individuals struggle to find nutritious foods. Several adulterants find their way into milk, such as detergents, water, cane sugar, rice flour, starch, formalin, hydrogen peroxide, sodium chloride, melamine, urea, cyanuric acid, vegetable oil, ammonium sulfate, caustic soda, boric acid, glucose, sorbitol, hypochlorite, salicylic acid and arrowroot [4]. Melamine is an organic industrial substance rich in nitrogen. The compound is NC-NH2, a trimer of cyanimide consisting of 67% nitrogen. C3H6N6 is the chemical composition of the

DOI: https://doi.org/10.54393/df.v6i1.163

compound. This systematic review and meta-analysis evaluates the levels, detection methods, and health risks of melamine and cyanuric acid in milk [5, 6]. Araujo et al., 2012 state that hydrolyzed melamine decomposes into ammelide, ammeline, and cyanuric acid [7]. It is commonly utilized in the manufacture of melamine resins through the reaction with formaldehyde [8]. Additionally, melamine is utilized in the production of plastics, decorative laminates, tableware, molding materials, coatings, flame retardant agents, and adhesives [9]. Besides its various industrial applications, melamine has seen growing utilization in food and dairy items as a synthetic protein source [10]. Due to its elevated nitrogen levels (66%), it is unlawfully included in baby formulas, animal feed, nitrogen fertilizers and milk products as an enhancer of protein [11]. The addition of melamine to packaged milk has become a widespread practice, as businesses employ it to boost market prices, enhance nitrogen levels in milk, and lower product expenses [12]. While melamine is safe in minimal amounts, a TDI (tolerable daily intake) threshold of 0.2 mg/kg has been established using dose-response evaluations from sub-chronic rat research. Additionally, safety thresholds of 1mg/kg melamine in baby formulas and 2.5mg/kg in other products have been set. Many countries have established Maximum Residue Limits (MRL) for melamine across various products to protect public health and ensure food safety. For example, the USFDA established the MRL of melamine in dairy products, milk foods and milk at 0.25 mg/kg, stressing that infant formula available to consumers of United States must be completely melamine-free, whereas the European Union (EU) set the MRL of melamine in dairy items and high-protein foods at 2.5 mg/kg [13, 14]. The present research can aid this effort in Pakistan by concentrating on the easy determination of melamine levels in milk products, marking an initial step toward regulating melamine in food items from local markets. Thus, the primary objectives of this investigation aimed to measure melamine levels and its associations between the protein concentrations of different packaging samples of milk and milk baby formulae with a foundation of local marketplaces in Pakistan.

Despite global regulatory standards for melamine in dairy products, routine surveillance and region-specific data on melamine contamination in Pakistan remain limited, particularly at the city level. Most previous investigations have either focused on advanced analytical techniques or broader national assessments, with insufficient comparative analysis between packed and unpacked milk available in local markets. Furthermore, there is a scarcity of recent empirical data from Lahore evaluating melamine levels using rapid and accessible methods such as ELISA. Therefore, localized evidence is essential to assess potential consumer exposure risks and support food safety monitoring frameworks. To accomplish these goals, ELISA based approach was employed to evaluate the presence of melamine in different milk items.

## METHODS

This experimental study employed the AgraQuant@ Melamine ELISA kit was used for the current experimental study. It contained melamine standard solutions (0, 20, 100 and 1000ppb), melamine enzyme conjugate, substrate solution, stop solution and wash buffer. ELISA microplate reader (Model: EZ Read 2000, biochrom), centrifuge machine, digital pipettes and microtubes, distilled water were also required for the test. Various samples of packed and unpacked milk (n=10 for packed and n=10 for unpacked) were collected randomly from different locations of Lahore. The unpacked milk samples were obtained from local market and milkmen providing milk at domestic levels in different regions of Lahore. The packed milk samples were collected from some highly recommended and commonly known supermarkets and stores from various locations in Lahore. The samples were kept in refrigerator after collection at -20ºC. 5mL of milk samples were pipetted out in tubes for centrifugation for 10 minutes at 1500rpm. The fat layer was removed and the clear milk serum was collected for analysis. 150μL of the melamine standards and samples were poured into antibody coated wells. 50μL of the conjugate was also added and mixed well by carefully pipetting up and down 3 times. These wells were incubated for 30 minutes at room temperature. The contents of the microwells were emptied and washed with diluted wash buffer solution for a total of 4 washes each. The wells were dried on multilayer absorbent paper towel. 100μL of the substrate solution was added to the wells and then kept for 20 minutes and room temperature. 100μL of the stop solution was added and noted the absorbance within 10 minutes at 450 nm by using an ELISA microplate reader [15]. A standard curve using the absorbance values of the melamine standards was constructed and calculated the melamine concentration in the milk samples based on the standard curve.

## RESULTS

LOD and LOQ of the ELISA kit method was 8.0μg/kg and 0.1-0.5mg/kg as prescribed on Kit brochure. Melamine levels were noted in mg/kg (ppm) with a set of 3 readings for each sample and made an average value out of it for each sample. Table 1 shows different readings of melamine observed under ELISA reader and their average value in mg/kg in unpacked milk samples.

**DOI:** https://doi.org/10.54393/df.v6i1.163

**Table 1:** Melamine Levels(mg/kg)in unpacked Milk Samples

| Samples | Value 1 (mg/kg) | Value 2 (mg/kg) | Value 3 (mg/kg) | Average (mg/kg) Mean ± SD |
|---|---|---|---|---|
| 1 | 6.2 | 6.4 | 6.3 | 6.30 ± 0.10 |
| 2 | 1.29 | 1.35 | 1.32 | 1.32 ± 0.03 |
| 3 | 1.46 | 1.52 | 1.49 | 1.49 ± 0.03 |
| 4 | 0.72 | 0.78 | 0.75 | 0.75 ± 0.03 |
| 5 | NOT DETECTED | | | |
| 6 | NOT DETECTED | | | |
| 7 | NOT DETECTED | | | |
| 8 | 0.56 | 0.62 | 0.59 | 0.59 ± 0.03 |
| 9 | 0.50 | 0.90 | 0.70 | 0.70 ± 0.20 |
| 10 | 0.13 | 0.19 | 0.16 | 0.16 ± 0.03 |

The results in figure 1 showed that sample 1 is unfit for human consumption as it has melamine beyond permissible levels of 2.5mg/kg.

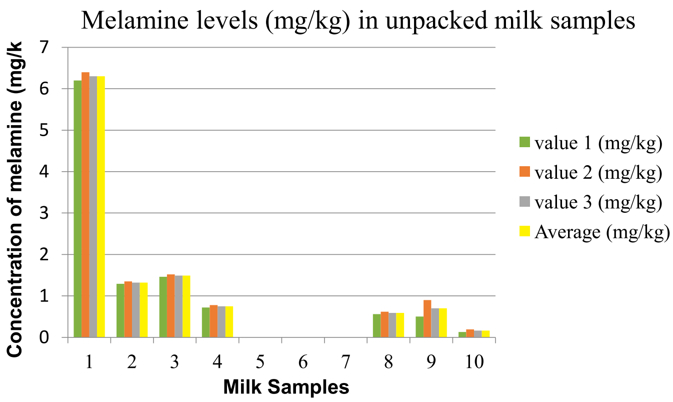


**Figure 1:** Comparison of melamine levels(mg/kg)in unpacked milk samples

Melamine concentrations detected in unpacked milk samples collected from different regions of Lahore (Table 2).

**Table 2:** Results of unpacked Milk Samples

| Samples | Melamine in unpacked samples (mg/kg) | Results |
|---|---|---|
| 1 | 6.30 | Not fit for human consumption |
| 2 | 1.32 | Fit for human consumption |
| 3 | 1.49 | Fit for human consumption |
| 4 | 0.75 | Fit for human consumption |
| 5 | 0 | Fit for human consumption |
| 6 | 0 | Fit for human consumption |
| 7 | 0 | Fit for human consumption |
| 8 | 0.59 | Fit for human consumption |
| 9 | 0.70 | Fit for human consumption |
| 10 | 0.16 | Fit for human consumption |

Although; the melamine contamination was found in other samples but that was within the safest permissible levels (Figure 2). However, sample 5, 6 and 7 was found with no melamine contamination.

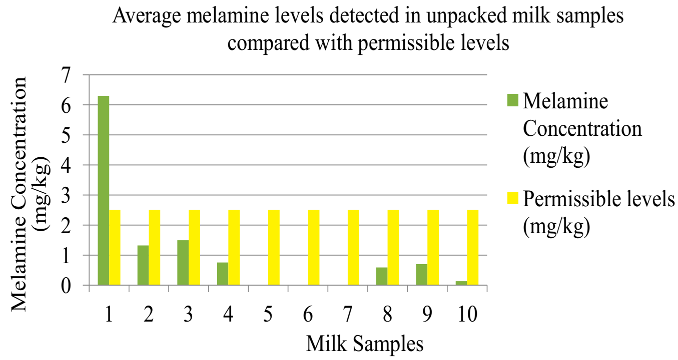


**Figure 2:** Comparison of unpacked Samples with Permissible Levels

3 readings of each sample were taken by ELISA reader at 450nm and calculated an average value out of it. Table 3 shows different readings of melamine in milk of packed samples. Figure 3 showed melamine levels (mg/kg) in 10 selected samples.

**Table 3:** Melamine levels(mg/kg)in packed samples

| Samples | Value 1 (mg/kg) | Value 2 (mg/kg) | Value 3 (mg/kg) | Average (mg/kg) Mean ± SD |
|---|---|---|---|---|
| 1 | 0.071 | 0.063 | 0.069 | 0.067 ± 0.004 |
| 2 | 0.168 | 0.175 | 0.164 | 0.169 ± 0.006 |
| 3 | 0.137 | 0.142 | 0.148 | 0.142 ± 0.006 |
| 4 | 0.13 | 0.127 | 0.124 | 0.127 ± 0.003 |
| 5 | 0.114 | 0.110 | 0.115 | 0.113 ± 0.002 |
| 6 | 0.118 | 0.123 | 0.122 | 0.121 ± 0.002 |
| 7 | NOT DETECTED | | | |
| 8 | 0.82 | 0.86 | 0.84 | 0.84 ± 0.020 |
| 9 | NOT DETECTED | | | |
| 10 | 0.37 | 0.33 | 0.35 | 0.35 ± 0.020 |

The permissible level or the level up to which the consumption of melamine in milk is safe is observed to be 2.5mg/kg by European Commission 2009[16].

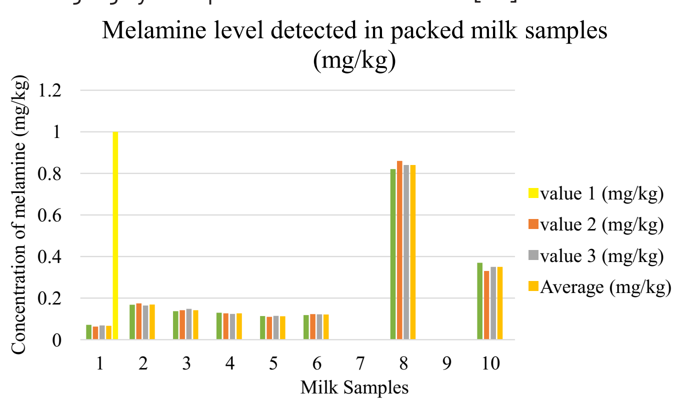


**Figure 3:** Comparison of Values of Packed Samples in mg/kg

By comparing packed milk samples with permissible levels of EU, 2009, it was noted that all milk samples were safe for human consumption (Table 4).

DOI: https://doi.org/10.54393/df.v6i1.163

**Table4:** Results of Packed Milk Samples

| Samples | Average level of Melamine in packed samples (mg/kg ) | Results |
|---|---|---|
| 1 | 0.067 | Fit for human consumption |
| 2 | 0.169 | Fit for human consumption |
| 3 | 0.142 | Fit for human consumption |
| 4 | 0.127 | Fit for human consumption |
| 5 | 0.113 | Fit for human consumption |
| 6 | 0.121 | Fit for human consumption |
| 7 | 0 | Fit for human consumption |
| 8 | 0.84 | Fit for human consumption |
| 9 | 0 | Fit for human consumption |
| 10 | 0.35 | Fit for human consumption |

Packed milk has lower levels of melamine as compared to the unpacked milk samples (Figure 4).

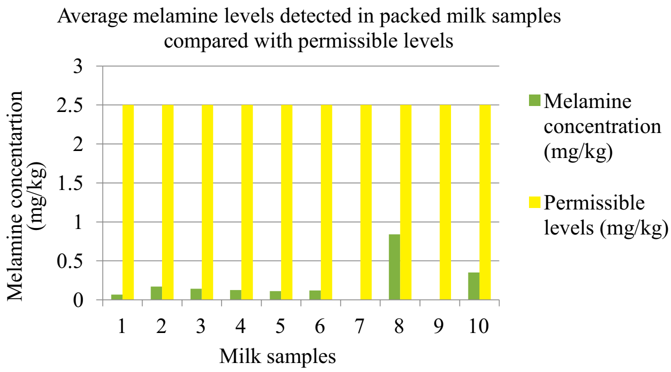


**Figure 4:** Comparison of packed samples with permissible level

Figure 5 depicted the comparison of melamine concentration in unpacked and packed milk samples alongwith permissible levels.

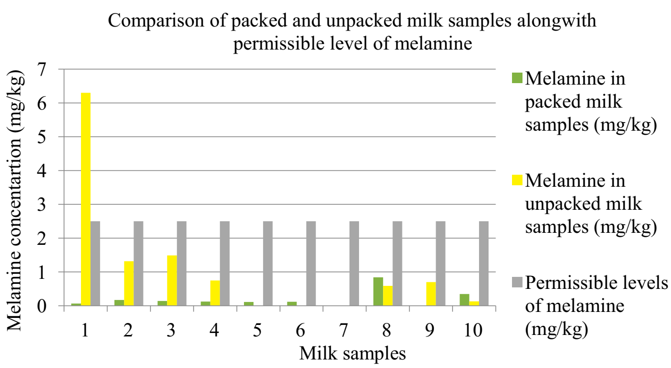


**Figure 5:** Comparison of Packed and Unpacked Milk Samples

## DISCUSSION

An article reviewed advancements in melamine detection, highlighting the evolution from traditional methods to innovative electrochemical sensor technologies [17]. Another study presented a rapid immunochromatographic assay using luminescent quantum dot beads for sensitive melamine detection in milk. [18]. In a study, 300 samples of milk and dairy products that were bought from well-known Turkish merchants were analyzed using the technique. While 2% of cheese, 8% of milk powder, and 44% of yogurt samples had melamine at levels of 121, 694 ± 146, and 294 ± 98 µg/kg, respectively, infant formulae and pasteurized UHT milk did not contain melamine. These results fell short of the thresholds established by EU law and the Codex Alimentarius Commission [18]. These results showed that packed samples were within permissible levels established by the Codex Alimentarius Commission (2010) and EU regulations (European Commission, 2002, 2009; 1 mg/kg for infant formula, 2.5 mg/kg for dairy goods)[19]. In a study conducted by Gouri, 2025, five distinct brands of protein powders underwent FTIR analysis, resulting in spectra for each sample. Eighty percent of the samples indicated the presence of melamine. To prevent melamine contamination and safeguard consumer safety, stringent quality control protocols and routine testing are recommended [20]. The contamination of one unpacked sample found beyond permissible levels is risky and smaller quantities found were due to contamination during the processing of dairy items, while the larger quantities were due to intentional inclusion. Eating foods with these minimal amounts of melamine poses no health risk to consumers. Despite progress, obstacles remain, such as the intricate and variable nature of milk composition, the significant expenses associated with advanced technology, the requirement for specialized knowledge, and the absence of uniform protocols [21].

This study was limited by a relatively small sample size and sampling restricted to selected areas of Lahore, which may limit generalizability to other regions of Pakistan. The use of a single analytical method (ELISA) without confirmatory techniques such as LC-MS/MS may also influence analytical precision. Additionally, seasonal variation and supply chain factors were not explored. Future research should include larger, multi-city surveillance studies using multiple validated analytical techniques to enhance accuracy and regulatory reliability. Strengthening routine monitoring systems and establishing standardized national screening protocols are recommended to ensure milk safety and protect public health.

## CONCLUSIONS

Melamine was absent in packaged milk samples and within the acceptable limits set by the European Commission in 2009; however, one sample of unpackaged milk revealed a significant presence of melamine. Additionally, some unpackaged milk samples showed minimal or no detectable levels of melamine. Therefore, this survey indicates that consumers of unpackaged milk face a certain risk of melamine exposure, while packaged milk presents a lower or negligible risk.

**DOI:** https://doi.org/10.54393/df.v6i1.163

## Authors' Contribution

Conceptualization: NZ
Methodology: MKS
Formal analysis: RJ, MS, SN
Writing and Drafting: AS, RJ, MS, SN
Review and Editing: AS, RJ, MS, SN, MKS, NZ

All authors approved the final manuscript and take responsibility for the integrity of the work.

## Conflicts of Interest

All the authors declare no conflict of interest.

## Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

## REFERENCES

[1] Saeed S, Rashid AA, Rizvi YA, Shehzad K, Nawaz S, Muhammad Y. Screening of melamine in Milk and Milk-based products using enzyme-linked immunosorbent assay. Nutrition and Food Sciences Research. 2021 Oct; 8(4): 29-36.
[2] Singh P and Gandhi N. Milk preservatives and adulterants: processing, regulatory and safety issues. Food Reviews International. 2015 Jul; 31(3): 236-61. doi: 10.1080/87559129.2014.994818.
[3] Siddiquee S, Saallah S, Bohari NA, Ringgit G, Roslan J, Naher L *et al.* Visual and optical absorbance detection of melamine in milk by melamine-induced aggregation of gold nanoparticles. Nanomaterials. 2021 Apr; 11(5): 1142. doi: 10.3390/nano11051142.
[4] Barham GS, Khaskheli M, Soomro AH, Nizamani ZA. Detection and extent of extraneous water and adulteration in milk consumed at Hyderabad, Pakistan. Journal of Food and Nutrition Sciences. 2014 Apr; 2(2): 47-52. doi: 10.11648/j.jfns.20140202.15.
[5] Abedini A, Hadian Z, Kamalabadi M, Salimi M, Koohy-Kamaly P, Basaran B *et al.* Melamine and cyanuric acid in milk and their quantities, analytical methods and exposure risk: A systematic review and meta-analysis. Journal of Food Protection. 2025 Feb; 88(3): 100454. doi: 10.1016/j.jfp.2025.100454.
[6] Filazi AY, Sireli UT, Ekici H, Can HY, Karagoz A. Determination of melamine in milk and dairy products by high performance liquid chromatography. Journal of Dairy Science. 2012 Feb; 95(2): 602-8. doi: 10.3168/jds.2011-4926.
[7] Araújo R, Moreira JL, Ratola N, Santos L, Alves A. Melamine and cyanuric acid in foodstuffs and pet food: method validation and sample screening. Analytical letters. 2012 Mar; 45(5-6): 613-24. doi: 10.1080/00032719.2011.649458.
[8] Hu Y, Feng S, Gao F, Li-Chan EC, Grant E, Lu X. Detection of melamine in milk using molecularly imprinted polymers-surface enhanced Raman spectroscopy. Food Chemistry. 2015 Jun; 176: 123-9. doi: 10.1016/j.foodchem.2014.12.051.
[9] World Health Organization. Toxicological and health aspects of melamine and cyanuric acid: report of a WHO expert meeting in collaboration with FAO, supported by Health Canada, Ottawa, Canada, 1-4 December 2008. InToxicological and health aspects of melamine and cyanuric acid: report of a WHO expert meeting in collaboration with FAO, supported by Health Canada, Ottawa, Canada, 1-4 December 2008 2009.
[10] Chu CY and Wang CC. Toxicity of melamine: the public health concern. Journal of Environmental Science and Health, Part C. 2013 Jan; 31(4): 342-86. doi: 10.1080/10590501.2013.844758.
[11] Yang L, Huo D, Jiang Y, Hou C, Zhang S. Monitoring the adulteration of milk with melamine: a visualised sensor array approach. Food Additives and Contaminants. Part A. 2013; 30(5): 786-95. doi: 10.1080/19440049.2013.793457.
[12] Zhang M, Ping H, Cao X, Li H, Guan F, Sun C et al. Rapid determination of melamine in milk using water-soluble CdTe quantum dots as fluorescence probes. Food Add Cont. Part A. 2012 Jan; 29(3): 333-44. doi: 10.1080/19440049.2011.643459.
[13] EFSA Panel on Contaminants in the Food Chain (CONTAM) and EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). Scientific opinion on melamine in food and feed. EFSA Journal. 2010 Apr; 8(4): 1573. doi: 10.2903/j.efsa.2010.1573.
[14] Hieu-Tran Q. Validation of the method for determination of melamine and investigation its trace in milk from Vietnam by LC-MS/MS. Asian Journal of Applied Chemistry. 2021 Feb; 8(1):13-9. doi: 10.9734/AJACR/2021/v8i130182.
[15] Li W, Meng M, Lu X, Liu W, Yin W, Liu J *et al.* Preparation of anti-melamine antibody and development of an indirect chemiluminescent competitive ELISA for melamine detection in milk. Food Agri Immunol. 2014 Oct; 25(4): 498-509. doi: 10.1080/09540105.2013.847063.
[16] European Commission (2009). Commission Regulation 1135/2009/EC of 25 November 2009 imposing special conditions governing the import of certain products originating in or consigned from China, and repealing Commission Decision 2008/798/EC. Off. J. L311:3. 2009 Nov; 52.
[17] Kalambate RP, Kalambate PK, Laiwattanapaisal W. Revolutionizing melamine detection: Cutting-edge

**DOI:** https://doi.org/10.54393/df.v6i1.163

advances from traditional analyses to state-of-the-art electrochemical sensors. Next Materials. 2024 Apr; 3: 100085. doi: 10.1016/j.nxmate.2023.100085.

[18] Chen Q, Qie M, Peng X, Chen Y, Wang Y. Immunochromatographic assay for melamine based on luminescent quantum dot beads as signaling probes. RSC Advances. 2020; 10(6): 3307-3313. doi: 10.1039/C9RA08350B

[19] Rambla-Alegre M, Peris-Vicente J, Marco-Peiró S, Beltrán-Martinavarro B, Esteve-Romero J. Development of an analytical methodology to quantify melamine in milk using micellar liquid chromatography and validation according to EU Regulation 2002/654/EC. Talanta. 2010 May; 81(3):894-900. doi: 10.1016/j.talanta.2010.01.034.

[20] Gouri SG. Detection of Melamine in Protein Powder. International Journal of Innovative Science and Research Technology. 2025 Apr 19; 10(4): 553-5. doi: 10.38124/IJISRT.

[21] Joolaei Ahranjani P, Dehghan K, Esfandiari Z, Joolaei Ahranjani P. A Systematic Review of Spectroscopic Techniques for Detecting Milk Adulteration. Critical Reviews in Analytical Chemistry. 2025 Mar 10:1-32. doi: 10.1080/10408347.2025.2477535.