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Study Review

A review of recent advances in melamine detection techniques

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ABSTRACT

Melamine is a nitrogen-rich chemical that has received much attention in recent years owing to a series of highly publicized food safety incidents. It is purposely added to foods by unethical manufacturers in order to elevate the organic nitrogen content, thus increasing the price and the profit made from such products. Recently, several methods have been established to determine melamine content. Unfortunately, most of these methods require complicated pre-concentration and costly instruments, and are time-consuming. Analytical procedures based on biosensors have emerged in scientific literature as a very promising alternative method due to their simplicity, speed and sensitivity. This review discusses current advances in detection techniques for melamine and its compounds. Current and past melamine contamination incidents, as well as modern instruments and analytical methods for determining the presence of melamine and its analogues are presented, and the characteristics of melamine and related compounds are also described.

Contents

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1. Introduction

Melamine is a nitrogen-rich heterocyclic triazine that is widely used in the synthesis of melamine formaldehyde resins (MFR) for the production of paper finishers, flame retardant, commercial filters, moulding compounds and wrinkle-free textile, as well as nanomaterials, since the 1950s (Mecker et al., 2012). Melamine has high nitrogen-rich content, ≈66% by mass. Accordingly, it has been illegally added to dairy products by unethical milk producers to obtain an incorrectly higher readout of apparent protein content than that determined by the conventional standard Kjeldahl test (Kim et al., 2010).

This has been a cause of serious diseases and many infants were intoxicated because the addition of melamine into food products can cause death. In 2007 contaminated pet food caused the death of house pets due to melamine-induced kidney failure. The melamine was added to the raw materials used to make the pet food in order to falsely increase the nitrogen content of the product. This case resulted in more than 5300 pet food products being recalled. After that, another case occurred in 2008, this time involving infants in China. In 2008, melamine was found to be a harmful additive in pet food, animal feed, milk and protein sources including wheat gluten, rice protein concentrate and corn gluten (Ehling et al., 2007; Mfiligenzi et al., 2008). In the Republic of China about 294,000 children became victims of melamine-tainted milk, and six deaths were reported (Zhou et al., 2011). Ping et al. (2012) reported that melamine and its metabolites are absorbed in the gastrointestinal tract and precipitate in the kidney to form crystals resulting in the formation of kidney stones.

Melamine by itself has low toxicity, but when it is combined with cyanuric acid it forms insoluble crystals which lead to kidney stones or kidney toxicity. The outbreak of the acute renal failure in cats and dogs in 2007 after the consumption of contaminated pet food suggested that the co-ingestion of melamine and cyanuric acid causes renal toxicity to occur (Brown et al., 2007; WHO, 2008a). Melamine cannot be metabolized by the body; approximately 90% of ingested melamine is excreted by the kidneys within 24 h. The distribution of melamine is likely limited to the body water fraction as melamine is unlikely to be bound in significant amounts to body tissues. When the amount of melamine ingested exceeds the excretion capability of the kidneys, renal disease and even death may occur.

The strong affinity between melamine and cyanuric acid results in the formation of an insoluble melamine–cyanurate complex through hydrolysis. In this process the hydrogen bonding causes crystallization, which leads to tissue injury including urolithiasis, bladder cancer and even death (Lee et al., 2011; Sun et al., 2011; Cao et al., 2010; Langman et al., 2009). In order to regulate melamine levels in food products and ensure national safety limits it is necessary to establish rapid, effective, and sensitive melamine detection methods.

The recent issues with melamine in food products have led to extensive and intensive laboratory monitoring work. Recently, various analytic methods have been developed and used to analyze the level of melamine in food samples. Several methods are included such as gas chromatography (GC) (Yokley et al., 2000), high performance liquid chromatography (HPLC) (Ehling et al., 2007), high performance liquid chromatography/mass spectrum (HPLC/MS) (Kim et al., 2008), surface enhanced Raman spectroscopy (Lin et al., 2008), capillary zone electrophoresis/mass spectrum (CE/MS) (Cook et al., 2005) and micellar liquid chromatography (MLC) (Beltran-Martinavarro et al., 2014). These methods are highly sensitive and specific, but also time-consuming, and they require costly instrumentation, extensive sample preparation and highly skilled personnel. On the other hand, enzyme-linked immunosorbent assay (ELISA) methods have the advantages of high throughput and rapid turnaround time in comparison to analytical instrumental methods. However, the procedure requires labour-intensive operations including incubation, washing and enzymatic reactions during the signal generation process. Thus, there is a need to develop simple, rapid, low-cost, convenient and highly sensitive methods to detect melamine in milk and dairy products.

Advances in chemistry, physics, biochemistry and molecular biology have led to the development of biosensors, which can detect a large range of biological elements with greater assay selectivity and sensitivity. The development of electrochemical biosensors for the detection of melamine is an important advance in melamine detection techniques owing to their sensitivity, selectivity, low cost, simplicity and possibility for miniaturization, portability and integration in automated devices (Farre et al., 2009). This electrochemical analytical technique is a viable alternative for determining lower concentrations of melamine in food and dairy products. Furthermore, only a small amount of sample is needed when using nanotechnology. So far these nanotechnologies have shown great potential for high throughput and rapid detection of melamine in developing technology (Sun et al., 2010a).

The goal of this review is to cover recent advances in melamine detection techniques. This review also discusses the physical and chemical structure of melamine, its applications and current and past melamine contamination incidents. An overview of the recent development of analytical methods for measuring melamine and its analogues in various tainted foods are presented. Analytical determination of melamine levels in food product is extremely important due to its toxicity to humans and animals, which has caused many countries around the world to establish strict controls for foods likely to be contaminated during harvest or storage.

2. Melamine: what it is, how it is used

2.1. Chemical structure

Melamine ($C_3H_6N_6$; MW: 126.12) is a small polar molecular compound that contains three amino groups that is found in the form of white crystals with high nitrogen levels (Liu et al., 2012). Interestingly, melamine can be hydrolyzed via deamination reactions and formed from cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine), ammeline (4,6-diamino-2-hydroxy-1,3,5-triazine), and ammelide (6-amino-2,4-dihydroxy-l,3,5-triazine) under strong acidic and alkaline conditions. Melamine and its analogues are also able to self-assemble in high molecular weight complexes organized by intra-molecular networks of hydrogen bonds and π–π aromatic ring stacking (Whitesides et al., 1991).

Cyanuric acid is an oxytriazine melamine analogue that may be produced as a by-product in melamine synthesis. It is a primary component in feed-grade biuret and a ruminant feed additive. Cyanuric acids derivatives are components in sanitizing solutions