

DETECTION OF MELAMINE IN PROTEIN POWDER

Gouri S G^{1}, Chandni T^{2**}

¹ Department of Forensic Science, St. Thomas College (Autonomous), Thrissur

² Assistant Professor, Department of Forensic Science, St. Thomas College (Autonomous), Thrissur

*Main author

**Co author

Abstract

Melamine is a nitrogenous compound often illicitly added to artificially inflate protein content, posing health risks. This fraudulent practice poses severe health risks, as melamine is non-digestible and can cause serious health issues, such as renal failure, kidney stones, and other complications, when consumed in large quantities. The study aims in the detection of melamine in protein powders using FTIR spectroscopy. 5 different brands of protein powders were subjected to the FTIR analysis and spectra were obtained for each sample. 80% of the samples tested positive for melamine. Rigorous quality control measures and regular testing are preferred to avoid melamine contamination and to ensure consumer safety.

Keywords: melamine, protein powder, FTIR analysis, spectral profile, adulteration

INTRODUCTION

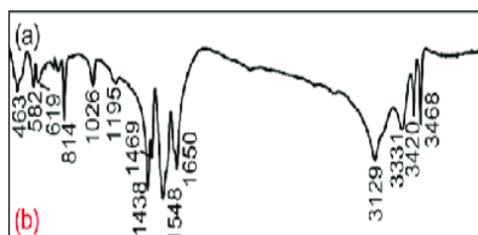
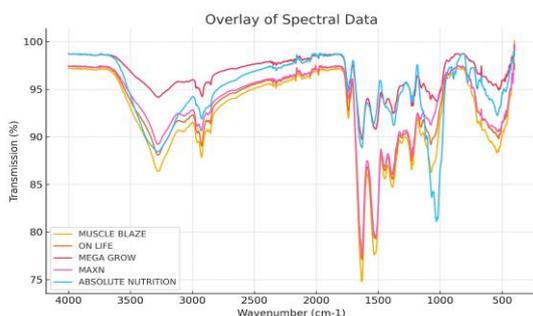
Protein powders are nutritional supplements that help to build muscle, repair tissue, make enzymes, hormones and also it aids in weight loss. Whey protein is a powdered form taken from whey, the liquid left over when cheese is made from cow's milk and whey protein is used to increase the protein in the diet. Isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, arginine, cystine, glycine, proline, tyrosine, serine, alanine, aspartic acid and glutamic acid are the main components in protein powders. Melamine (C₃H₆N₆) is a nitrogen rich white crystalline powder used in the manufacturing of plastics, coatings, adhesives, filters and containers. As melamine is not natural, it is not approved for direct addition to food or feed. By artificially inflating the nitrogen content, melamine has been used to falsely elevate the apparent protein levels in various food items including milk, milk powder and infant formula. This poses severe health risks such as renal failure, kidney stones and other complications. The aim of the study is to detect melamine from whey protein powders using Fourier transform infrared (FTIR) spectroscopy.

MATERIALS AND METHOD

Five protein powder samples of different brands were collected. No sample preparation was required prior to analysis. The detection of melamine in these samples was carried out using Fourier Transform Infrared Spectroscopy (FTIR). The spectral profiles of all the samples were obtained and the resulting spectral peaks were then matched against the standard spectral peaks of melamine to confirm its presence. The FTIR used was Perkin Elmer Spectrum Two, Model LI6000 A. The wavelength ranges from 8300 cm⁻¹ to 350 cm⁻¹ with the use of KBr beam splitter for better performance. The spectral resolution is 0.5 cm⁻¹.

RESULTS

Upon analysis, the spectral peaks of samples were compared with the standard spectral peaks of melamine. In sample 1, 2, 4 and 5, characteristic peaks of melamine were observed. Also in samples 2, 4 and 5 additional peaks indicate the impurities present. Sample 3 showed no significant melamine peaks, indicating little to no presence of melamine. The results indicate that melamine contamination is present in the majority of samples.



(i) overlay of spectra of samples

(ii) standard spectra of melamine

Standard IR absorption bands of melamine

| wavelength (cm ⁻¹) | functional group | type of vibration |
|--------------------------------|------------------|-------------------|
| 3450 - 3100 | N-H | Stretching |
| 1650 - 1550 | C=N | Stretching |
| 1550 - 1350 | Triazine ring | Deformation |
| 1350 - 1100 | C=N | Stretching |
| 810 - 820 | Triazine core | Ring vibration |

IR absorption bands of samples

| Sample | N-H(cm ⁻¹) | C=N(cm ⁻¹) | Triazine | C-N(cm ⁻¹) | N-H(cm ⁻¹) | Melamine |
|--------|------------------------|------------------------|----------|------------------------|------------------------|----------|
| 1 | 3278.6 | 1631.2 | 1520.4 | 1333, 1266.5 | - | Present |
| 2 | 3270.9 | 1626 | 1519.6 | 1332.8 | - | Present |
| 3 | - | - | 1631.2 | 1003.0 | - | Absent |
| 4 | 3277.5 | 1630 | - | 1529 | 812.7 | Present |
| 5 | 3278.3 | 1631.0 | - | 1532 | - | Present |

1. OBSERVATION

- In sample 1, the peaks at 3278.6 cm⁻¹ (N-H), 1631.2 cm⁻¹(C=N), 1520.4 cm⁻¹(triazine) and 1333 cm⁻¹ strongly indicate the presence of melamine.
- In sample 2, the peaks 3270.9 cm⁻¹(N-H), 1626.0(C=N), 1519.6 cm⁻¹(triazine ring) and 1332.8 cm⁻¹ (C-N) indicates the presence of melamine but the presence of the peak 1737.5 cm⁻¹ indicates the presence of other impurities.
- In sample 3, the absence of strong 3270-3300 cm⁻¹(N-H) peak and the missing 1330-1260 cm⁻¹(C-N) peak suggests little to no melamine content.
- In sample 4, the peaks around 3277 cm⁻¹(N-H), 1630 cm⁻¹(C=N), 1529 cm⁻¹(C-N) and 812 cm⁻¹ (N-H bending) strongly indicate that melamine is present. The additional peaks at 2924.7 cm⁻¹ and 1740.8 cm⁻¹ indicate the presence of impurities.
- In sample 5, the peaks 3278.3 cm⁻¹(N-H), 1631 cm⁻¹(C=N), 1532cm⁻¹ indicates that melamine is likely present but the absence of a clear N-H bending peak (~810 cm⁻¹) suggests either a low concentration or interference with other compounds. Also additional peaks 2925.1 cm⁻¹ and 1741.2 cm⁻¹ indicate the presence of impurities.

DISCUSSION AND CONCLUSION

Melamine is a nitrogenous compound often illicitly added to food products to artificially inflate the protein content. Since melamine is not natural, it is not approved for direct addition to food. Melamine cause serious health issues like renal failure, kidney stones and other complications. As it is concerning, a comprehensive study was undertaken to investigate the presence of melamine in whey protein powder. Five protein powder samples from distinct brands were randomly selected and subjected to Fourier Transform Infrared Spectroscopy analysis. The FTIR spectra of the samples were carefully examined and compared with standard melamine spectral peaks to ascertain the presence of melamine. The results revealed that four out of the five samples tested positive for melamine, indicating a significant concern for consumer safety. While this study focused on detection, future research can prioritize quantification of melamine concentrations using advanced chromatographic techniques.

REFERENCE

- [1] Anthony Kai-ching Hau, Tze Hoi Kwan, Philip Kam-tao Li, 2009, Melamine Toxicity and the Kidney, *Journal of the American Society of Nephrology* 20 (2), 245-250
- [2] Carl G Skinner, Jerry D Thomas, John D Osterloh, 2010, Melamine Toxicity, *Journal of Medical Toxicology* 6, 50-55
- [3] Catherine Berthomieu, Rainer Hienerwadel, 2009, Fourier Transform Infrared Spectroscopy, *Photosynthesis research* 101, 157-170
- [4] Céline Marie-Elise Gossner, Jørgen Schlundt, Peter Ben Embarek, Susan Hird, Danilo Lo-Fo-Wong, Jose Javier Ocampo Beltran, Keng Ngee Teoh, Angelika Tritscher, 2009, The Melamine Incident: Implications for International Food and Feed Safety, *Environmental health perspectives* 117 (12), 1803-1808
- [5] Chenghui Lu, Bingren Xiang, Gang Hao, Jianping Xu, Zhengwu Wang, Changyun Chene, 2009, Rapid detection of melamine in milk powder by near infrared spectroscopy, *Journal of Near Infrared Spectroscopy* 17 (2), 59-67
- [6] Elis angela do Carmo Domingo, Aline Auxiliadora Tirelli, Cleiton Antonio Nunes, Mario César Guerreiro, Sandra Maria Pint, 2013, Melamine Detection In Milk Using Vibrational Spectroscopy And Chemometric Analysis : A Review, *Food Research International* 60, 131-139
- [7] Fengxia Sun, Wei Ma, Liguang Xu, Yinyue Zhu, Liqiang Liu, Chifang Peng, Libing Wang, Hua Kuang, Chuanlai Xu, 2010, Analytical methods and recent developments in the detection of melamine, *TrAC Trends in Analytical Chemistry* 29 (11), 1239-1249
- [8] Harini Uppada, Jogaratnam G, Santu B, Rushika K, Jagadeesh Panda, 2024, A Comprehensive Review on Melamine: Insights into Risks and Detection Techniques, *International Journal of Pharmaceutical Sciences Review and Research*
- [9] Kobun Rovina, Shafiqzaman Siddiquee, 2015, A review of recent advances in melamine detection techniques, *Journal of Food Composition and Analysis* 43, 25-38
- [10] Lisa J. Mauer, Alona A. Chernyshova, Ashley Hiatt, Amanda Deering, Reeta Davis, 2009, Melamine Detection in Infant Formula Powder Using Near- and Mid-Infrared Spectroscopy, *Journal of agricultural and food chemistry* 57 (10), 3974-3980
- [11] Mena Ritota, Pamela Manzi, 2017, Melamine Detection in Milk and Dairy Products: Traditional Analytical Methods and Recent Developments, *Food analytical methods* 11, 128-147
- [12] Na Guan, Qingfeng Fan, Jie Ding, Yiming Zhao, Jingqiao Lu, Yi Ai, Guobin Xu, Sainan Zhu, Chen Yao, Lina Jiang, Jing Miao, Han Zhang, Dan Zhao, Xiaoyu Liu, Yong Yao, 2009, Melamine-Contaminated Powdered Formula and Urolithiasis in Young Children, *New England Journal of Medicine* 360 (11), 1067-1074
- [13] Roman M. Balabin, Sergey V. Smirnov, 2011, Melamine detection by mid- and near-infrared (MIR/NIR) spectroscopy: A quick and sensitive method for dairy products analysis including liquid milk, infant formula, and milk powder, *Talanta* 85 (1), 562-568
- [14] Rose Saxton, Owen M McDougal, 2021, Whey Protein Powder Analysis by Mid-Infrared Spectroscopy, *Foods* 10 (5), 1033
- [15] Sana Jawaid, Farah N Talpur, Hassan Imran Afridi, Shafi M Nizamani, Abid A Khaskheli, Saba Naz, 2014, Quick determination of melamine in infant powder and liquid milk by Fourier Transform Infrared spectroscopy, *Analytical Methods* 6 (14), 5269-5273
- [16] Sana Jawaid, Farah N Talpur, Sherazi, Shafi M Nizamani, Abid A Khaskheli, 2013, Rapid detection of melamine adulteration in dairy milk by SB-ATR–Fourier transform infrared spectroscopy, *Food chemistry* 141 (3), 3066-3071
- [17] Tsung-Hsuan Tsai, Soundappan Thiagarajan, Shen-Ming Chen, 2010, Detection of Melamine in Milk Powder and Human Urine, *Journal of agricultural and food chemistry* 58 (8), 4537-4544
- [18] Vivek Bhalla, Paul C Grimm, Glenn M Chertow, Alan C Pao, 2009, Melamine nephrotoxicity: an emerging epidemic in an era of globalization, *Kidney international* 75 (8), 774-779
- [19] Wenting Liang, Yuqiang Wei, Mengjie Gao, Xin Yan, Xinhua Zhu, Wenchuan Guo, 2020, Detection of Melamine Adulteration in Milk Powder by Using Optical Spectroscopy Technologies in the Last Decade—a Review, *Food Analytical Methods* 13, 2059-2069
- [20] Yang Lu, Yinqiang Xia, Guozhen Liu, Mingfei Pan, Mengjuan Li, Nanju Alice Lee, Shuo Wang, 2016, A review of methods for detecting melamine in food samples, *Critical reviews in analytical chemistry* 47 (1), 51-66
- [21] Yuan Liu, Ewen ED Todd, Qiang Zhang, Jiang-rong Shi, Xian-jin Liu, 2012, Recent developments in the detection of melamine, *Journal of Zhejiang University Science B* 13 (7), 525-532