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Advanced Glycation Risk in Diabetic Nutritional Supplements: GC-MS-Based Quantification of key Dicarbonyl Compounds

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ABSTRACT

Methylglyoxal (MGO) and glyoxal (GO) are highly reactive α-dicarbonyl compounds implicated in numerous pathological conditions and frequently formed during food processing and storage. Due to their potential health risks, especially among diabetic populations, precise quantification in widely consumed protein supplements is critical. This study presents a validated, sensitive gas chromatography/mass spectrometry (GC/MS) method utilizing 3,3',5,5'-tetramethylbenzidine (TMB) for derivatization of MGO and GO in various protein supplements, including diabetes-specific formulations. The developed method exhibited outstanding linearity ($R^2 \geq 0.999$), low detection limits (GO: 0.005 µg/mL, MGO: 0.01 µg/mL), and high recovery rates (85–95%). Commercial diabetes care protein powders demonstrated significantly elevated MGO (2.93–4.14 µg/mL) and GO (22.36–32.40 µg/mL) levels, while raw protein powders contained the lowest concentrations. The data emphasize the impact of processing on dicarbonyl formation and underscore the necessity of regular monitoring to ensure consumer safety and product integrity.

Keywords: *Methylglyoxal, Glyoxal, Protein Supplements, GC/MS, TMB Derivatization, Diabetes Nutrition, Food Safety*

1. INTRODUCTION

Alpha-dicarbonyls such as glyoxal (GO) and methylglyoxal (MGO) are toxic intermediates formed during thermal processing and storage of food products. These compounds, through their high reactivity, modify proteins and nucleic acids, resulting in the formation of advanced glycation end-products (AGEs), which are closely associated with aging, diabetes, neurodegenerative disorders, and cardiovascular diseases.[1-2] Additionally, GO and MGO have demonstrated mutagenic and genotoxic properties in biological systems.[3].

A primary mechanism underlying the toxicity of methylglyoxal (MGO) and glyoxal (GO) is their high reactivity with essential biological macromolecules, particularly proteins and DNA. These α-dicarbonyl compounds readily react with free amino groups of lysine and arginine residues, as well as thiol groups of cysteine residues in proteins, resulting in the formation of advanced glycation end-products (AGEs). MGO, in particular, is recognized as a potent precursor in AGE formation. The accumulation of AGEs contributes to protein dysfunction, elevated oxidative stress, and chronic inflammation—key pathological features linked to age-related diseases and diabetic complications. Moreover, both MGO and GO have been shown to induce DNA damage, including strand breaks and mutations, thereby exerting cytotoxic and potentially mutagenic effects.[4-9]]

In food matrices, these dicarbonyls originate from several chemical reactions, including the Maillard reaction, sugar caramelization, and lipid peroxidation, as well as enzymatic reactions in fermented foods. Given the popularity of protein supplements—particularly those designed for individuals with diabetes—evaluating the concentrations of MGO and GO in these products is crucial for ensuring safety and quality.[10-13]

While several analytical techniques exist for quantifying MGO and GO, including HPLC, capillary electrophoresis, and LC/MS, derivatization is often required due to their small molecular sizes and poor volatility. This study adopts 3,3',5,5'-tetramethylbenzidine (TMB) as a derivatizing agent for efficient GC/MS analysis, producing distinct quinoxaline derivatives that enable accurate quantification.[14-19]

Dietary intake from various food sources contributes to the overall body burden of methylglyoxal (MGO) and glyoxal (GO). While the body has detoxification mechanisms, primarily the glyoxalase system, to manage these dicarbonyl compounds, chronic or excessive exposure can overwhelm these protective systems, resulting in the accumulation of MGO and GO and the subsequent formation of harmful advanced glycation end products (AGEs). For individuals with diabetes, who already exhibit elevated endogenous levels of these compounds, additional intake from dietary sources such as protein supplements could significantly increase their burden of reactive dicarbonyls, potentially exacerbating their health risks.[20]

Individuals with diabetes are particularly vulnerable to the toxic effects of MGO and GO due to their impaired glucose metabolism, which leads to increased endogenous production of these compounds, and the reduced efficacy of their detoxification pathways. Consequently, additional dietary exposure to MGO and GO from protein supplements could heighten their risk of developing or worsening diabetic complications. Notably, a study has suggested that higher habitual intake of MGO is associated with reduced low-grade inflammation but also with impaired retinal venular dilation, highlighting the complex and potentially contradictory health outcomes of dietary MGO intake.[21]

A review of the provided research shows a lot of information on the toxicity, formation, and presence of methylglyoxal (MGO) and glyoxal (GO) in biological systems and food. Many of the studies focus on the connection between these compounds and diabetes-related issues. There is also a lot of information on how MGO and GO form in foods during cooking or processing, and how GC/MS is used to analyze them. [21-24]However, none of the studies directly measure the levels of MGO and GO in protein supplements, including those designed for diabetes care. While there is data on MGO and GO in other foods like cookies, fried foods, oils, honey, and drinks, there is no specific information on protein supplements in the research provided. One study focuses on measuring the protein content in protein powders using a method called Bradford's Assay and RP-HPLC, but it doesn't measure MGO or GO. Another study talks about the general concerns with protein supplements, such as contamination with heavy metals or pesticides, and the nutritional benefits of whey protein, but doesn't mention MGO or GO levels. Some research discusses the biological roles and harmful effects of MGO and GO in diseases but doesn't look at their levels in protein supplements. One study mentions the presence of GO and MGO in UHT milk (a dairy product used to make whey protein), but it doesn't provide data on the protein powder itself.[25-27]

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents Standard solutions of GO and MGO (40% w/v) and TMB were acquired from Sigma-Aldrich. Standards for quinoxaline and 2-methylquinoxaline, along with HPLC-grade methanol and ultrapure water, were utilized throughout the study.

2.2 Instrumentation and GC/MS Conditions Analyses were carried out using an Agilent 7890B GC coupled with a 5977A mass spectrometer. Separation was performed on an HP-5MS capillary column (30 m × 250 µm i.d., 0.25 µm film). The oven was programmed from 100°C (2 min hold) to 250°C at 20°C/min, with a final hold at 250°C for 5 min. SIM mode was employed using m/z 130 and 103 for GO and m/z 144 and 117 for MGO.

2.3 Sample Preparation Raw protein supplements were precipitated with methanol and centrifuged; the supernatant was reacted with TMB at 60°C for 3 hours. Diabetic portein Powdered samples underwent a similar process after reconstitution in methanol. All derivatized samples were dried under nitrogen and reconstituted in methanol for GC/MS analysis.

2.4 Quantification and Calibration Calibration curves were created using serial dilutions of quinoxaline and 2-methylquinoxaline (0.2–100 µg/mL). LOD and LOQ values were determined based on signal-to-noise ratios (3:1 and 10:1, respectively).

2.5 Method Validation Precision was assessed through intra- and inter-day analyses, while recovery studies were conducted using spiked samples. Acceptable reproducibility was indicated by RSD values below 20%.

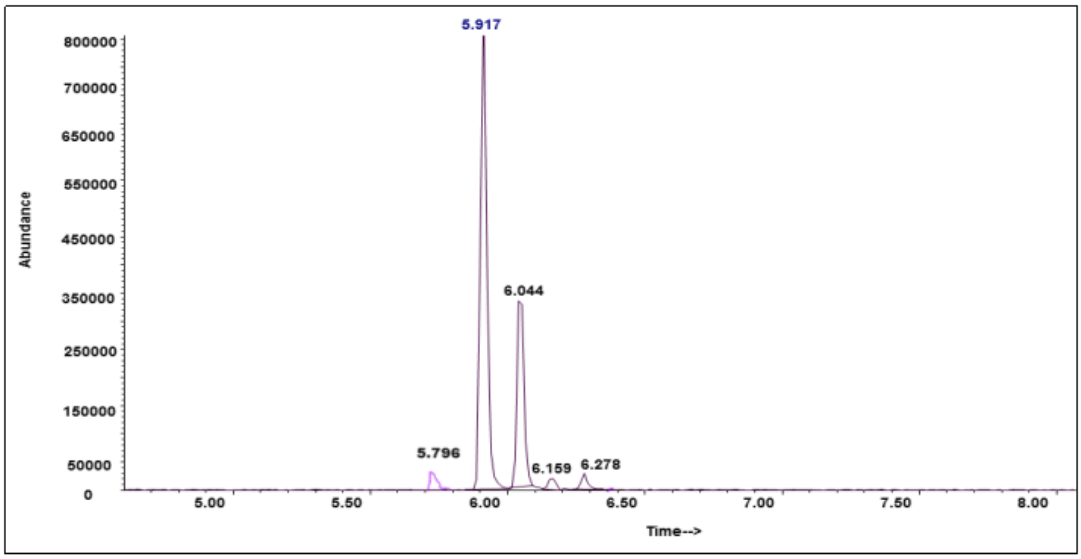


Figure 1: showing five isomers formed after the reaction of MGO with methloxyamine in GC/MS Extracted ion chromatogram (EIC) of *m/z* 117.

3. RESULTS AND DISCUSSION

3.1 Derivatization and Chromatographic Optimization Compared to other derivatizing agents, TMB generated singular, well-resolved peaks for both GO and MGO, facilitating clean and efficient chromatographic separation. The retention times for MGO and GO derivatives were ~5.70 min and ~4.98 min, respectively.

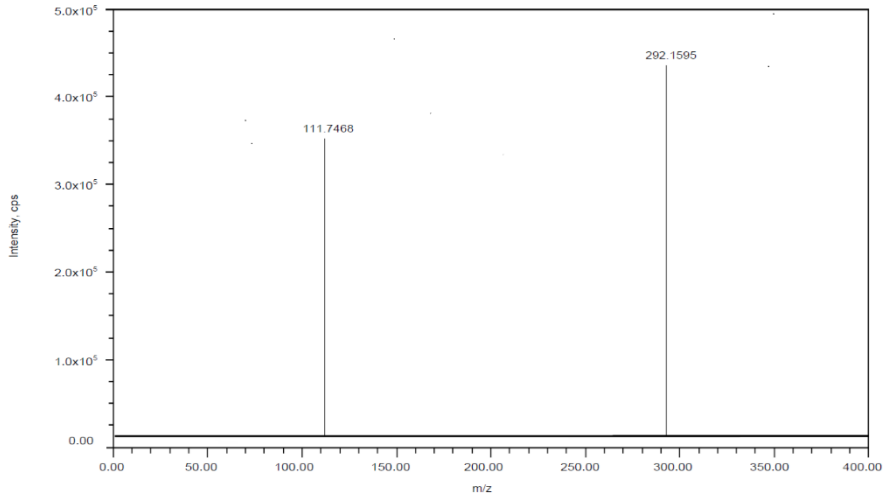
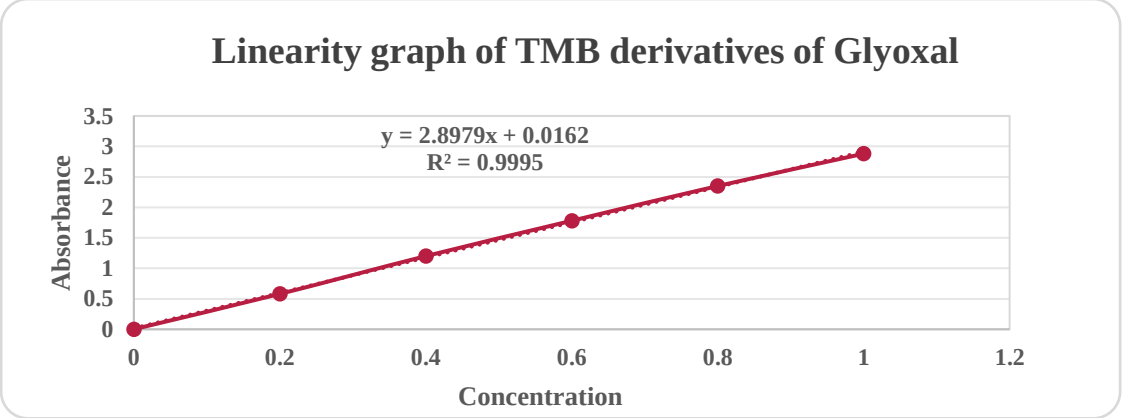


Figure-2: Mass spectra of GO and MGO at m/z=1117.468 and 292.1595

3.2 Analytical Performance Calibration demonstrated high linearity ($R^2 \geq 0.999$), and the method exhibited low LODs (MGO: 0.01 µg/mL; GO: 0.005 µg/mL) and LOQs (0.02 µg/mL for both), indicating strong method sensitivity and specificity.



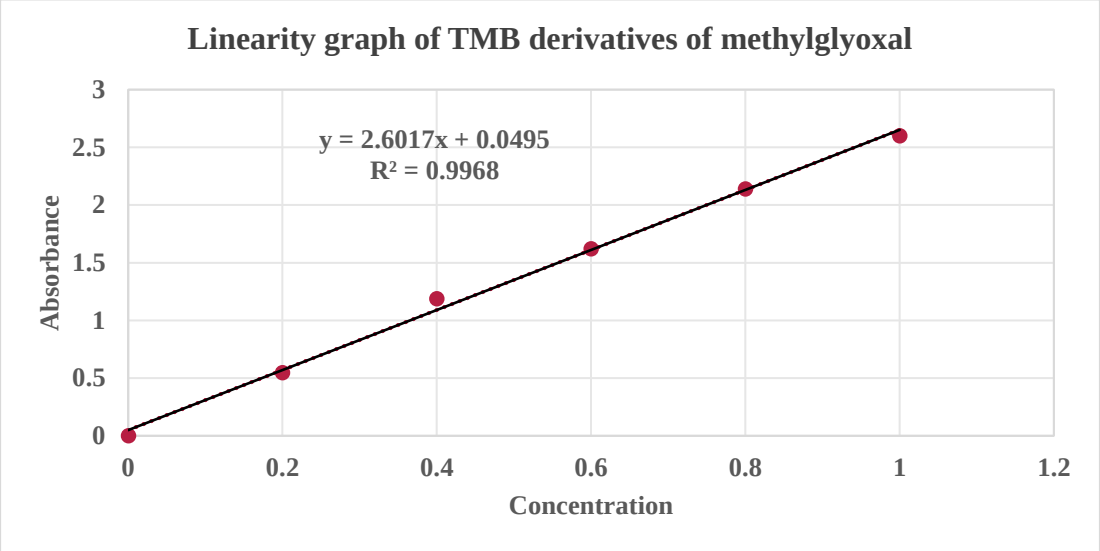


Figure-3: Linearity graph of TMB derivatives of Glyoxal and methylglyoxal

Compound	Linear range (µg/ml)	Regression equation	Correlation coefficient (R^2)	LOD (µg/ml)	LOQ (µg/ml)
GO	0.2-100	y = 2.8979x + 0.0162	0.9995	0.005	0.02
MGO	0.2-100	y = 2.6017x + 0.049	0.9968	0.01	0.02

Table 1.Calibration data and Limit of quantification (LOQ) and Limit of detection of the derivatives of GO and MGO.

3.3 Dicarbonyl Content in Supplements GO and MGO concentrations varied significantly across sample types. Commercial diabetes care protein powders contained the highest levels, likely due to extensive processing. Powdered protein supplements recorded the lowest dicarbonyl levels, highlighting the impact of minimal processing.

3.4 Method Validation Results Intra- and inter-day precision analyses yielded RSDs under 20%, while recovery ranged from 90–95% for MGO and 85–90% for GO, confirming the reliability and reproducibility of the methodology.

4. REGULATORY LIMITS AND QUALITY STANDARDS FOR MGO AND GO IN FOOD

Currently, there are generally no established international or broad national regulatory limits for the toxic dicarbonyl compounds methylglyoxal (MGO) and glyoxal (GO) in food products, including protein supplements, which places a significant responsibility on manufacturers to ensure product safety and quality through careful ingredient sourcing and processing. In the absence of mandatory regulations, the establishment of voluntary quality standards by the food supplement industry or individual manufacturers is crucial, particularly with manufacturers of diabetes care protein powders being advised to consider implementing stringent internal quality control standards for MGO and GO that are significantly lower than typical levels in general processed foods. Given that GC/MS is a suitable analytical technique for quantifying these compounds and that current literature lacks specific data on their levels in protein supplements, manufacturers are urged to prioritize thorough testing of both raw ingredients and finished products, optimize processing methods to minimize formation, exercise caution in ingredient selection, and consider establishing stringent internal quality control standards; consumers, especially those with diabetes, should be aware of potential MGO and GO intake from various foods, including supplements, and consider choosing transparent manufacturers, while future research should focus on quantifying levels, investigating influencing factors, determining safe intakes, and exploring mitigation strategies.

5. CONCLUSION

In conclusion, this research successfully developed and validated a highly sensitive and reliable GC/MS method, leveraging TMB derivatization, for the precise quantification of MGO and GO in a diverse range of protein supplements, including formulations specifically designed for diabetes care. The method's exceptional analytical performance was confirmed through rigorous validation, demonstrating outstanding linearity ($R^2 \geq 0.999$) across the tested concentration ranges, low limits of detection (LOD) of 0.005 µg/mL for GO and 0.01 µg/mL for MGO, and high recovery rates ranging from 85% to 95%. This robust validation establishes the method's suitability for routine monitoring and quality control. The stark contrast in dicarbonyl content observed, with significantly elevated levels of MGO (2.93–4.14 µg/mL) and GO (22.36–32.40 µg/mL) in processed commercial diabetes care protein powders compared to the lowest concentrations found in raw protein sources, unequivocally highlights the substantial impact of manufacturing processes on the formation of these potentially harmful compounds. These findings underscore a critical need for stringent monitoring and optimization of processing techniques within the protein supplement industry, particularly for products targeting vulnerable populations such as individuals with diabetes, who are already predisposed to elevated levels of these reactive species. Ultimately, the validated methodology and the presented data serve as a vital tool for manufacturers and regulatory bodies alike, enabling informed assessments of food safety and facilitating the development of strategies to minimize dicarbonyl content, thereby safeguarding consumer health and ensuring product integrity in this increasingly popular nutritional sector.

REFERENCES

[1] Methylglyoxal - Wikipedia [Internet]. [cited 2025 May 1]. Available from: https://en.wikipedia.org/wiki/Methylglyoxal

[2] GLYOXAL | - Ataman Kimya [Internet]. [cited 2025 May 1]. Available from: https://atamankimya.com/sayfalar.asp?LanguageID=2&cid=3&id=12&id2=1904

[3] safety issues of methylglyoxal and potential scavengers [Internet]. [cited 2025 May 1]. Available from: https://journal.hep.com.cn/fase/EN/10.15302/J-FASE-2017174

[4] Methylglyoxal, the dark side of glycolysis - PMC [Internet]. [cited 2025 May 1]. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4321437/

[5] Molecular Assessment of Methylglyoxal-Induced Toxicity and Therapeutic Approaches in Various Diseases: Exploring the Interplay with the Glyoxalase System - MDPI [Internet]. [cited 2025 May 1]. Available from: https://www.mdpi.com/2075-1729/14/2/263

[6] Methylglyoxal, a Highly Reactive Dicarbonyl Compound, in Diabetes, Its Vascular Complications, and Other Age-Related Diseases | Physiological Reviews [Internet]. [cited 2025 May 1]. Available from: https://journals.physiology.org/doi/full/10.1152/physrev.00001.2019

[7] The role of methylglyoxal and the glyoxalase system in diabetes and other age-related diseases - PubMed [Internet]. [cited 2025 May 1]. Available from: https://pubmed.ncbi.nlm.nih.gov/25818485/

[8] Safety issues of methylglyoxal and potential scavengers - ResearchGate [Internet]. [cited 2025 May 1]. Available from: https://www.researchgate.net/publication/326848468_Safety_issues_of_methylglyoxal_and_potential_scavengers

[9] Quantitative Analyses of Glyoxal and Methylglyoxal Compounds in French Fry Samples by HPLC Using 4-Nitro - DergiPark [Internet]. [cited 2025 May 1]. Available from: https://dergipark.org.tr/tr/download/article-file/1310087

[10] Analysis, Distribution, and Dietary Exposure of Glyoxal and Methylglyoxal in Cookies and Their Relationship with Other Heat-In - DergiPark [Internet]. [cited 2025 May 1]. Available from: https://digital.csic.es/bitstream/10261/54917/3/OPEN_SCI_085.pdf

[11] Gas chromatographic analysis of glyoxal and methylglyoxal formed from lipids and related compounds upon ultraviolet irradiation | Journal of Agricultural and Food Chemistry - ACS Publications [Internet]. [cited 2025 May 1]. Available from: https://pubs.acs.org/doi/10.1021/jf00026a016

[12] Analysis, Distribution, and Dietary Exposure of Glyoxal and Methylglyoxal in Cookies and Their Relationship with Other Heat-Induced Contaminants | Request PDF - ResearchGate [Internet]. [cited 2025 May 1]. Available from: https://www.researchgate.net/publication/41406664_Analysis_Distribution_and_Dietary_Exposure_of_Glyoxal_and_Methylglyoxal_in_Cookies_and_Their_Relationship_with_Other_Heat-Induced_Contaminants

[13] Advanced Glycation End Products: A Comprehensive Review of Their Detection and Occurrence in Food - PMC [Internet]. [cited 2025 May 1]. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10253085/

[14] Gas Chromatograph-Mass Spectrometry - Shimadzu [Internet]. [cited 2025 May 1]. Available from: https://www.shimadzu.com/an/products/gas-chromatograph-mass-spectrometry/index.html

[15] Gas Chromatography-Mass Spectrometry (GC-MS) Principle, Instrument, Detectors, and Combining Mass Spectrometers with other Techniques [Internet]. [cited 2025 May 1]. Available from: https://cmro.in/index.php/jcmro/article/view/866

[16] Learn About GC/MS Analysis - Innovatech Labs [Internet]. [cited 2025 May 1]. Available from: https://www.innovatechlabs.com/newsroom/642/stuff-works-gcms-analysis/

[17] What Is the Principle Behind GC-MS | MtoZ Biolabs [Internet]. [cited 2025 May 1]. Available from: https://www.mtoz-biolabs.com/what-is-the-principle-behind-gc-ms.html

[18] Gas Chromatography Mass Spectrometry (GC-MS) Information - Thermo Fisher Scientific [Internet]. [cited 2025 May 1]. Available from: https://www.thermofisher.com/us/en/home/industrial/mass-spectrometry/mass-spectrometry-learning-center/gas-chromatography-mass-spectrometry-gc-ms-information.html

[19] Gas chromatography–mass spectrometry - Wikipedia [Internet]. [cited 2025 May 1]. Available from: https://en.wikipedia.org/wiki/Gas_chromatography%E2%80%93mass_spectrometry

[20] Habitual intake of dietary methylglyoxal is associated with less low-grade inflammation: the Maastricht Study - PubMed Central [Internet]. [cited 2025 May 1]. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9761753/

[21] Habitual intake of dietary methylglyoxal is associated with less low-grade inflammation: the Maastricht Study - PubMed [Internet]. [cited 2025 May 1]. Available from: https://pubmed.ncbi.nlm.nih.gov/36055771/

[22] Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms [Internet]. [cited 2025 May 1]. Available from: https://pubs.acs.org/doi/10.1021/acs.jafc.7b00882

[23] The role of methylglyoxal and the glyoxalase system in diabetes and other age-related diseases. | Semantic Scholar [Internet]. [cited 2025 May 1]. Available from: https://www.semanticscholar.org/paper/The-role-of-methylglyoxal-and-the-glyoxalase-system-Maessen-Stehouwer/ed09b72d0a2fbc8d9449878a3a561ff7d78fcffb

[24] Glyoxal (Cicads 57, 2004) - InChem.org [Internet]. [cited 2025 May 1]. Available from: https://www.inchem.org/documents/cicads/cicads/cicad57.htm

[25] An Effective Derivatization Method for Quantitative Determination of Glyoxal and Methylglyoxal in Plasma Samples by Gas Chromatography/Mass Spectrometry - PubMed - NCBI [Internet]. [cited 2025 May 1]. Available from: https://www.ncbi.nlm.nih.gov/m/pubmed/12672144

[26] QUANTIFYING AMOUNT OF WHEY PROTEIN IN VARIOUS PROTEIN POWDERS [Internet]. [cited 2025 May 1]. Available from: https://www.westmont.edu/sites/default/files/2024-09/Gifty%20Blankson%20Codjoe_final.pdf

[27] A human health risk assessment of heavy metal ingestion among consumers of protein powder supplements - PMC [Internet]. [cited 2025 May 1]. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7509468/