Detection of Dye **Adulteration** in Botanical Products

The Role of NMR Spectroscopy

Arun Krishnamurthy, Ph.D.

NMR Spectroscopist

150 Research Lane, Suite 102
Guelph ON, N1G4T2, Canada

info@purity-iq.com

www.purity-iq.com

The Most Active Ingredient in Any Product is **TRUST**

Introduction

Among many food adulteration practices, the spiking of herbs, spices and natural health products with dyes to mimic their vibrant colours is quite common. Colourants provide no nutritional value and are often added to mask defects in foods or enhance their appearance. In some cases, the addition of dyes can be detrimental to consumer health. Dyes used in adulteration can be broadly classified into two categories:

1. Originating from a botanical source [ex. anthocyanins (E163), betalains (E162), riboflavin (E101), curcumin (E100), carotenoids (E160, E161), and starch-based processed colourants like caramels (E150)]

2. Synthetic dyes (ex. malachite green), and azo dyes (ex. metanil yellow, orange II, sudan red-I, sudan-red-G, sudan red-IV, rhodamine B, and azorubine, etc.).

Several studies have investigated dyed foods and their health consequences. One such study highlights that an alarming number of notification reports from Rapid Alert System for Food and Feed (RASFF) within the European Union pertain to contamination of chilli pepper (Capsicum annum) and tomato (*Solanum lycopersicum*) with the above-listed synthetic dyes [1]. Similarly, a 2005 product recall by the Government of Canada identified several chilli powder products to be adulterated with Sudan dyes [2]. A recent review identifies the widespread practice of palm oil adulteration with Sudan dyes in many developing countries, where much of the world's palm oil originates [3]. Even confectionaries and bakery products are not spared, a research article identified the adulteration of bakery products with metanil yellow and malachite green [4]. Furthermore, natural food colorants such as anthocyanin- and betalain-based coloring agents have been adulterated with synthetic textile dyes like Reactive Red 195 [5]. These examples represent only a fraction of the significant dye-related food adulteration issues prevalent in the market.

Synthetic dyes are associated with serious health risks are of great concern to consumers and the food industry as a whole. They are mutagenic, teratogenic, carcinogenic, and can induce chromosomal abnormalities, physiological and neurobehavioral changes, and disrupt spermatogenesis. Clinical studies show that malachite green and its major metabolite leucomalachite green induce the formation of DNA adducts and *cII* transgene mutations in mouse liver [6]. Sudan dyes are equally dangerous [7], and the German Federal Institute for Risk Assessment (BfR) classifies Sudan dyes as category 2 carcinogens and recommends that these should be included in stringent monitoring measures as no safe consumption level for Sudan dyes has been established. [8]. Similarly, metanil yellow is also a harmful dye with severe adverse effects to the nervous, cardiovascular, and digestive systems [9,10].

Synthetic dyes are prominent in industries like textiles, plastic, and rubber but often find their way into food products due to inadequate regulatory guidelines and analytical testing capabilities, warranting highly sensitive and accurate analytical techniques. Effective analytical methods for detecting dye adulteration should typically include the **following features:**

01 Botanical species authentication

02 Identification of product irregularities

03 Accurate detection and quantification

04 High Intra- and inter-lab reproducibility

05 Easy and straightforward data analytics to facilitate database building

Nuclear Magnetic Resonance (NMR) spectroscopy, with its non-targeted metabolomic profiling capability and inherent quantitative nature, is an ideal technique for such applications. NMR has gained prominence in recent years as a tool to identify and screen metabolites due to its high reproducibility, accuracy, ease of use and simple sample preparation methods. The unique metabolites in plant extracts yield distinctive NMR spectroscopic profiles, making NMR-based metabolomics an attractive and traceable approach for botanical identification. Additionally, NMR's capability to quantify molecules of interest in a mixture allows simultaneous detection and quantification of adulterants and dyes in plant extracts. NMR analytics are versatile and can be employed in authenticating raw materials, finished products, extracts, formulations, and oils of botanical origin. This versatility positions NMR as a comprehensive solution for manufacturers and vendors with diverse product portfolios. The methods can also be optimized to include synthetic dye adulteration and quantification steps.

As the title of this note states, *identifying harmful dyes in botanical products should not be a game of dice and left to chance. It requires testing methods that can deliver highly precise and accurate results.* This note provides several examples of food adulteration with various dyes and demonstrates the effective use of NMR-based methods for detecting such adulteration.

Materials and Methods

Samples Used in This Study

All samples were extracted using a suitable solvent and ^{1}H NMR data were acquired on a 400 MHz Bruker® NMR spectrometer. Hierarchical Clustering Analysis was performed, and the calculated Euclidean distances of the samples were plotted as a circular dendrogram for visualization.

Paprika (*Capsicum annum*)
Sample was spiked with sudan red-I, sudan red-G and sudan red-7B.

Turmeric (*Curcuma longa*)
Sample was spiked with metanil yellow.

Beet (*Beta vulgaris*)
Grape (*Vitis vinifera*)
Extracts were spiked with black-rice extract.

Results

Botanical extracts spiked with synthetic dyes and natural colourants are difficult to differentiate as they are visually alike. For example, paprika, turmeric, grape and beet extracts with different fractions of sudan-I, metanil yellow and black-rice extracts look alike and cannot be identified visually (**Figure 1**). Many conventional techniques fail to reliably detect dyes due to limitations such as detection limits, failure to differentiate molecules due to common functional groups (e.g., infrared spectroscopy), labour-intensive sample preparation and extraction methods, or require multiple testing protocols to identify both the botanical species and the dyes that are present. Taking these into consideration, NMR spectroscopy-based analytics offers a simple solution to identify dye adulteration.
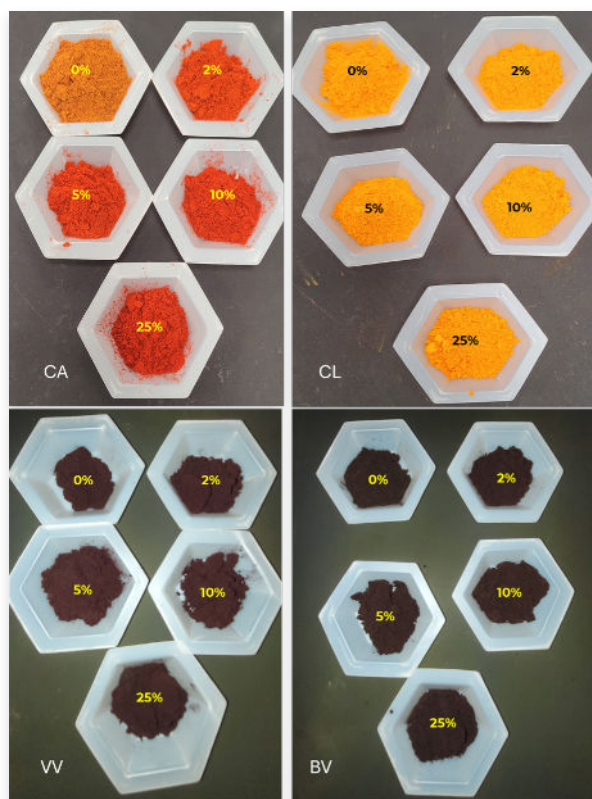

Figure 1: Pictures of paprika (*Capsicum annum*, CA), turmeric (*Curcuma longa*, CL), grape (*Vitis vinifera*, VV), and beet (*Beta vulgaris*, BV) extracts with different fractions of sudan-I, metanil yellow, and black-rice extracts, respectively.
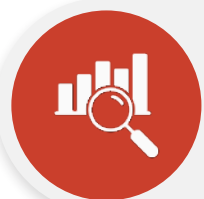
The ¹H NMR spectra of pure and dye-adulterated samples are plotted in **Figure 2** and **Figure 3**, which capture key chemical attributes of the test samples:

- ✓ Almost all synthetic dyes comprise aromatic functional groups and the aromatic region of the test sample spectrum captures the presence and identity of the synthetic dyes.

- ✓ As the ¹H NMR peak profile of the dyes is driven by their chemical structures and are unique, the spectral analysis accurately identifies the dye present, which applies to the natural colourants as well.

- ✓ The intensity of the dyes' peaks is characteristic of their concentrations; hence the extract of dye spiking can be accurately determined.

- ✓ The overall spectral profile of the botanical extract aids in the authentication of the botanical species. As the synthetic dyes primarily comprise aromatic functional groups, their resonances are restricted to the high frequency region (6-10 ppm), and the rest of the profile will be identical to that of an authentic botanical extract.

- ✓ The NMR spectral profiles of adulterated samples can be added to the existing NMR spectral library (database), aiding in the analysis of new adulterated samples in the future. This also allows for a robust library building, comprising several common dye adulterants and their respective botanical targets.
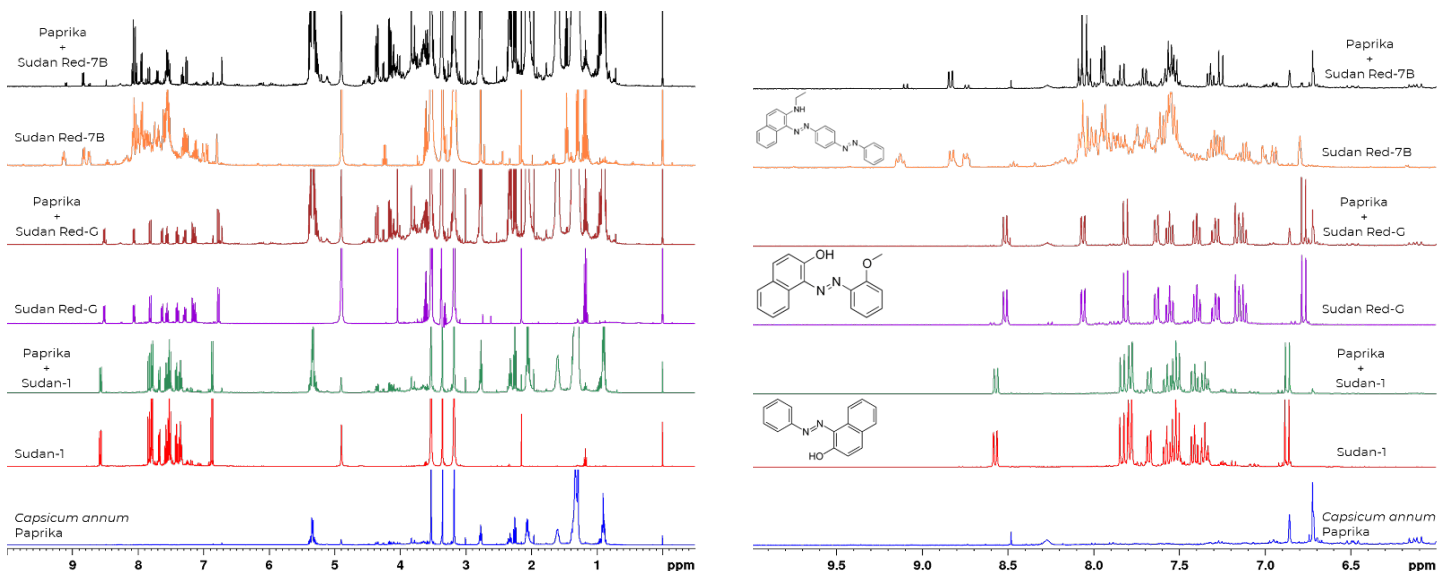

Figure 2: ¹H NMR profiles of sudan dyes and paprika extract spiked with these dyes: Left – Complete spectral profile; Right – Aromatic region.
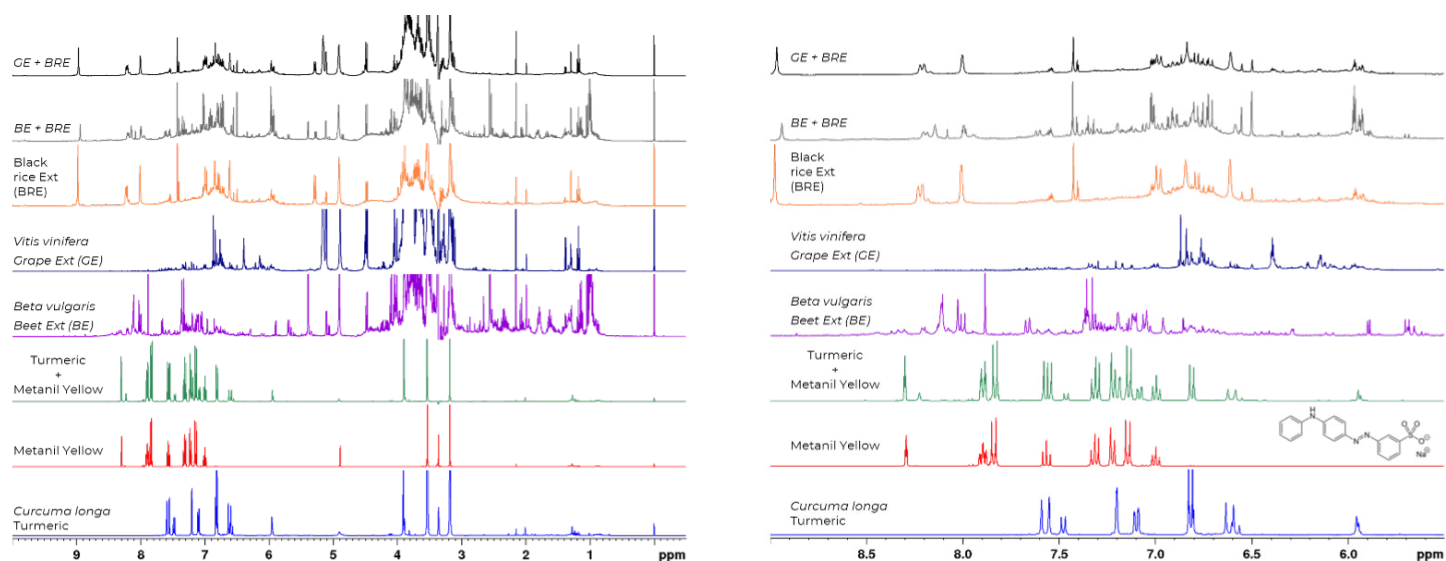
Figure 3: ^{1}H NMR profiles of turmeric with metanil yellow, grape, and beet extracts with black-rice extract (BRE). Left – Complete spectral profile; Right – Aromatic region.

The spectral changes caused by the addition of dyes to botanical extracts are significant enough to be observed statistically as well. The circular dendrogram built on Euclidean distances through Hierarchical Clustering Analysis clearly demonstrates the spectroscopic differences between authentic and dye-spiked samples (**Figure 4**). Authentic samples of turmeric, paprika, grape and beet extracts cluster into distinct groups separate from the dye-spiked samples. Using this dataset as a spectral library and statistical model, new test samples can be applied against this to predict the true chemical nature and dye adulteration. Moreover, by using supervised statistical models such as the Linear Discriminant Analysis (LDA), trained models can be built with classified data clusters (**Figure 5**), which will confidently predict the authenticity of the products. These statistical methods combined with the visual screening of the spectral data offer multi-level scrutiny of the analytical data, making NMR-based analytics highly accurate and reliable.
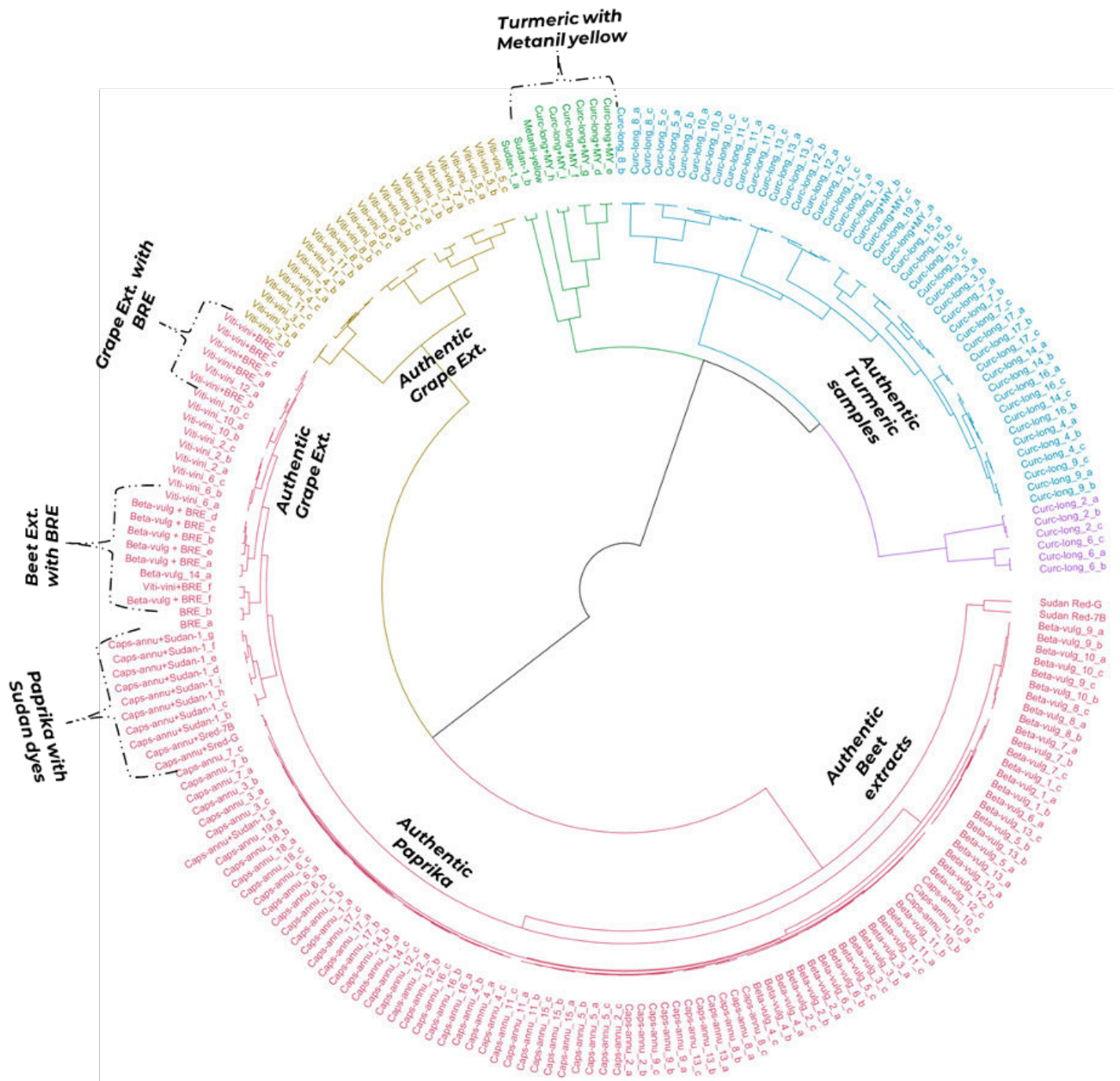
Figure 4: Circular dendrogram from Hierarchical Clustering Analysis differentiating pure and dye-spiked botanical samples. A subset of authentic sample data from the spectral library is shown for clarity.
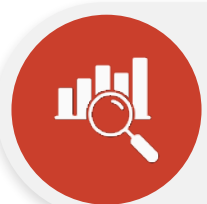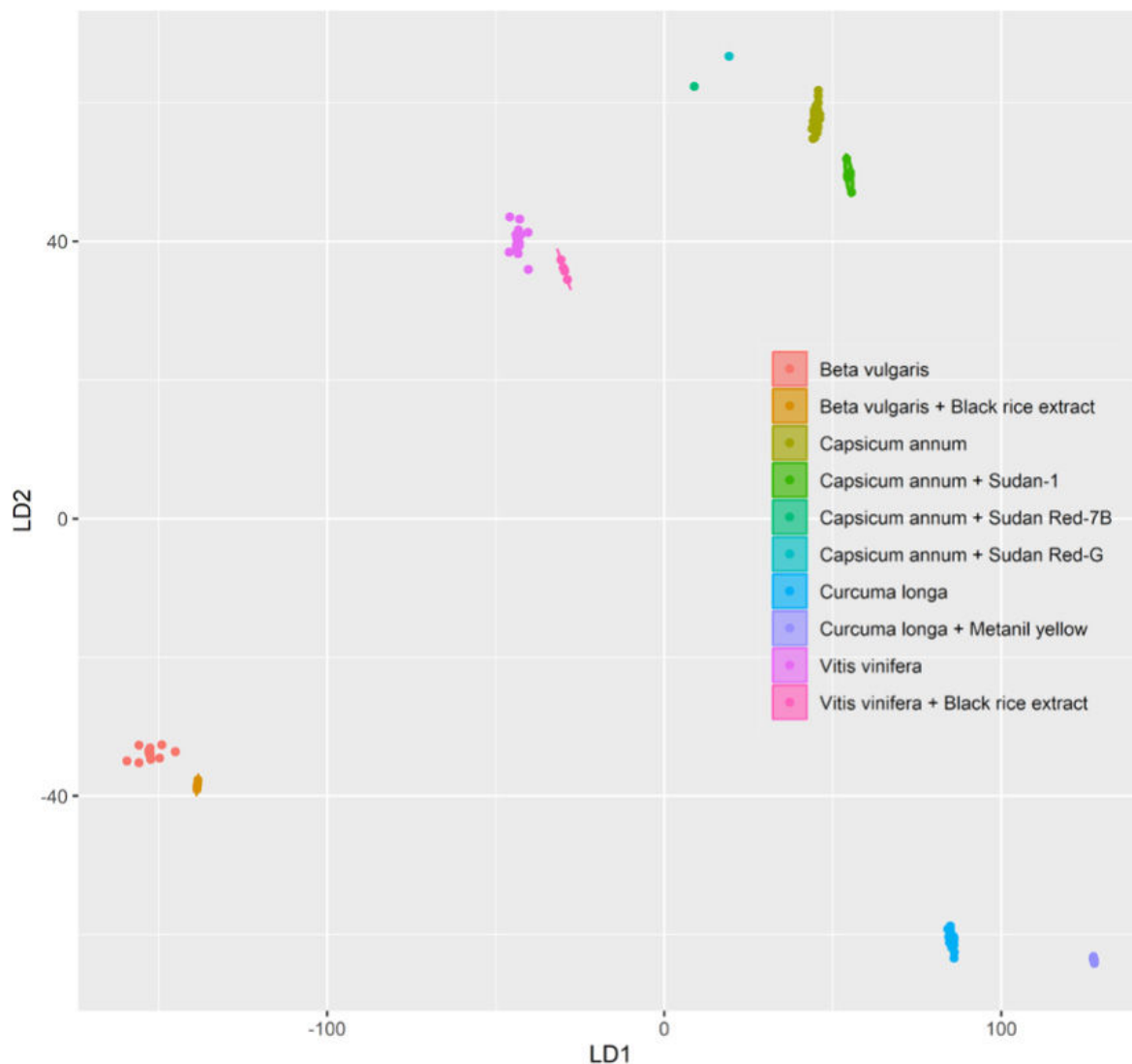
Figure 5: Linear Discriminant Analysis plot showing the differentiation of pure and dye-adulterated datasets into different clusters.

Conclusions

The data presented showcases the high-level chemical insights achievable through NMR spectroscopy. This approach can be further applied to identify and quantify a wide range of synthetic and natural dyes commonly found in the market. Product authenticity, including species identification, quality assessment, adulteration detection, and batch-to-batch consistency, can be integrated into a single analysis performed simultaneously, ensuring no detail is left to chance.

References

1. E.K. Essuman, E. Teye, R.G. Dadzie, L.K. Sam-Amoah, Pesticide Residues and Unauthorized Dyes as Adulteration Markers in Chilli Pepper and Tomato, International Journal of Food Science, 1 (2023), 5337150.

2. CFIA-Canada, Update: Health hazard alert – certain food products may contain sudan dyes https://www.canada.ca/en/news/archive/2005/02/update-health-hazard-alert-certain-food-products-may-contain-sudan-dyes.html, February 25, 2005 (retrieved on November 21, 2024).

3. R. Nyorkeh, E. Teye, E.K. Essuman, S.A. Haughey, N. Logan, C.T. Elliott, 18 Year analysis of RASFF notifications on Sudan dye adulterated cases in palm oil (2004 to 2022) and the principles of the technique for its detection, Sustainable Food Technology, 2(5) (2024) 1235-1245.

4. D. Singh Yadav, S. Jaiswal, Analysis of non-permitted dyes in bakery and dairy products for forensic consideration, International Journal of development research, 6(7) (2016) 8775-8779.

5. J. Müller-Maatsch, R.M. Schweiggert, R. Carle, Adulteration of anthocyanin- and betalain-based coloring foodstuffs with the textile dye "Reactive Red 195" and its detection by spectrophotometric, chromatic and HPLC-PDA-MS/MS analyses, Food Control, 70 (2016) 333-338.

6. Alexander J, Barregård L, Bignami M, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Knutsen HK. Malachite green in food. Efsa Journal. 2016 Jul 27 (https://doi.org/10.2903/j.efsa.2016.4530).

7. Fonovich TM. Sudan dyes: are they dangerous for human health? Drug and chemical toxicology, 36(3) (2013) 343-52.

8. Federal Institute for Risk Assessment (BfR) Dyes sudan I to IV in food, BfR opinion of 19 November 2003 (Retrieved on November 21, 2024) (www.bfr.bund.de/cm/349/dyes_sudan_I_IV.pdf.)

9. D. Ghosh, P.S. Singha, S.B. Firdaus, S. Ghosh, Metanil yellow: The toxic food colorant, Asian Pacific Journal of Health Sciences 4 (2017) 65–66.

10. A. Tiwari, Toxic effect of metanil yellow ss food adulterant: A Review, Think India Journal, 22 (2019) 1652.