APPLICATION NOTE
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CURCUMA SPECIES IDENTIFICATION AND ADULTERANT DETECTION USING NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY – A CASE STUDY FOR BOTANICALS

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Abstract

The global demand for turmeric has sky-rocketed in recent years due to its therapeutic properties, resulting in severe adulteration of turmeric dietary supplements with other curcuma species of lower economic values and low curcuminoid concentrations. In addition, the turmeric supplements are spiked with azo- and inorganic-dyes to enhance the aesthetic appearance of the product and with fillers such as corn and cassava starch to increase the volume of the product. In this report, we demonstrate the capability of Nuclear Magnetic Resonance spectroscopy, in conjunction with multivariate statistical analysis, in identifying curcuma species, determining the purity of the products, detecting curcuma adulterants, and highlighting the advantages of using NMR as a quality control tool in the analysis of botanical products.

Introduction

Curcuma longa, an herbaceous plant commonly known as turmeric is in the limelight for its use primarily in the food industry, cosmetics, and in traditional medicine due to its therapeutic properties and its use to treat biliary disorders, anorexia, hepatic disorder, pain, inflammation, and other ailments [1,2]. Curcuminoids are the bioactive components of turmeric, responsible for the anti-diabetic, anti-inflammatory, antioxidant, antibacterial, analgesic, neuroprotective, cardioprotective, and anti-hyperlipidemia properties of turmeric and turmeric-based dietary supplements. Due to growing demand for turmeric and turmeric-based dietary supplements, adulteration of turmeric rhizome powder and extracts has become prolific, with the adulterants being **the herbs from the same genus** such as *Curcuma mangga*, *Curcuma amada*, *Curcuma zedoaria*, *Curcuma caesia*, and, *Curcuma aromatica*, which have a lower economic value and poor curcumin concentrations [3,4], **synthetic curcumin**, dyes such as **metanil yellow**, lead chromate and Sudan red are used to mimic the visual appearance of the product, and fillers like **corn** and **cassava starch** are used to increase the product volume, all of which underscores the complexity of the adulteration issue.

Nuclear Magnetic Resonance (NMR) spectroscopy has gained prominence in recent years as a tool to identify and screen the metabolites constituting plant extracts due to its high reproducibility, accuracy, ease of use and simple sample preparation methods. The metabolites constituting plant extracts are unique to each species and yield distinctive NMR spectroscopic profiles, making metabolomics by NMR an attractive and traceable approach for botanical identification. In addition, the capability of NMR to quantify specific molecules in a mixture ascribes a quantification aspect to the analysis. The molecule-screening attribute of NMR offers the unique advantage of detecting, identifying, and quantifying adulterants present in a sample in a single analysis. Coupling NMR with multivariate statistical analysis of the data further enhances the screening capabilities of the technique.

As the metabolomic profile of samples are unique to each type, NMR proves to be a robust tool and functions as a fingerprinting technique to test the consistency between samples from different batches (i.e., batch-to-batch consistency) in the manufacturing sector, and to establish the purity and origin of raw materials used in the production of consumer products. In this report, we demonstrate the utility of NMR in identifying the curcuma species and adulterants common to turmeric.

Materials and Methods

Curcuma longa, *Curcuma amada*, *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma aromatica* plant products were obtained from different raw-material suppliers. Corn starch, cassava starch, synthetic curcumin, natural curcumin extract, and metanil yellow were procured commercially. Metabolites from these plant products were extracted using different solvents such as water, methanol, chloroform, acetone, ethanol, hexane, and their mixtures. The solvent system that rendered the highest concentration of both polar and non-polar metabolites was adopted for this study. One-dimensional NMR spectra were collected on a 400 MHz Bruker Avance III spectrometer with tetramethyl silane (TMS, 0 ppm) as an internal chemical shift reference. The collected spectra were processed, baseline and phase corrected, and binned using Bruker Amix program for multivariate statistical analysis, performed on the R platform.

Results and Discussion

The ^{1}H NMR spectra of Curcuma mangga, Curcuma caesia, cassava starch, Curcuma longa, natural curcumin, synthetic curcumin, and metanil yellow are plotted in ***Figure 1***. Significant differences are observed upon comparing these spectra, attributed to the distinct metabolite array constituting these plant extracts, which differ in their composition, i.e., the types of polar and non-polar metabolites and their concentration. The position of the peaks in the spectrum, their splitting patterns, and the correlation between the peaks, which is probed using two-dimensional NMR experiments, serve as a tool to identify the exact metabolites present in the extract. Furthermore, the intensity of the peaks is directly proportional to the concentration of the metabolite they represent, hence the metabolites can be quantified accurately.

Several spectra were collected for each product type by analyzing multiple samples, the spectral ensemble was used in building a library for statistical analysis, the result of which is shown in ***Figure 2***. Due to the differences in the metabolite profile and their concentration, the samples belonging to the same species and adulterants cluster in different regions of the linear discriminant analysis plot. The spectra of three test samples, two Curcuma longa, and one natural curcumin extract, were fit into the model, and we observe that they align with their respective clusters, therefore attesting to the accuracy of the developed model for species identification.

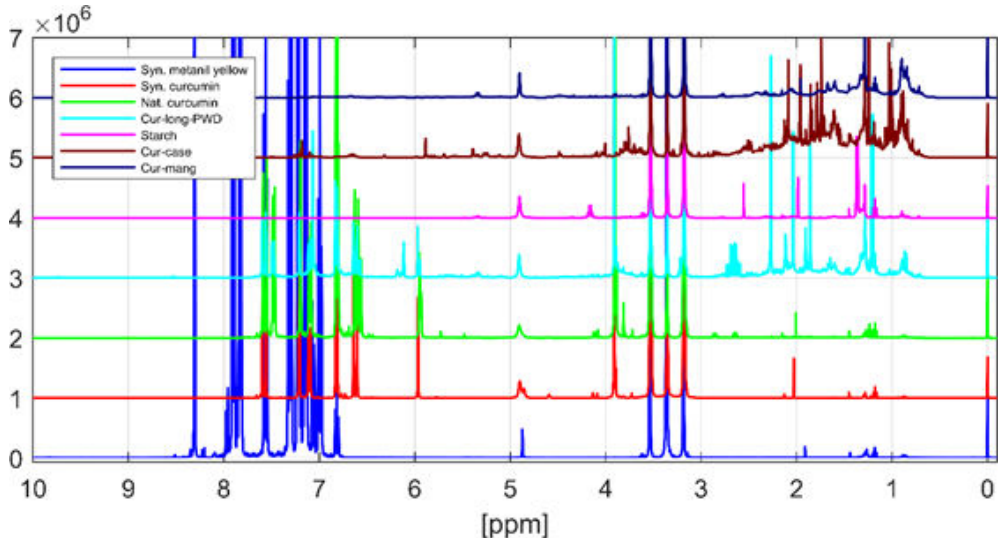

Figure 1. An overlay of the ^{1}H NMR spectra of *Curcuma mangga* (Cur-mang), *Curcuma caesia* (Cur-case), cassava starch (starch), *Curcuma longa* (Cur-long-PWD), natural curcumin (Nat. curcumin), synthetic curcumin (Syn.curcumin), and metanil yellow (Syn.metanil yellow).

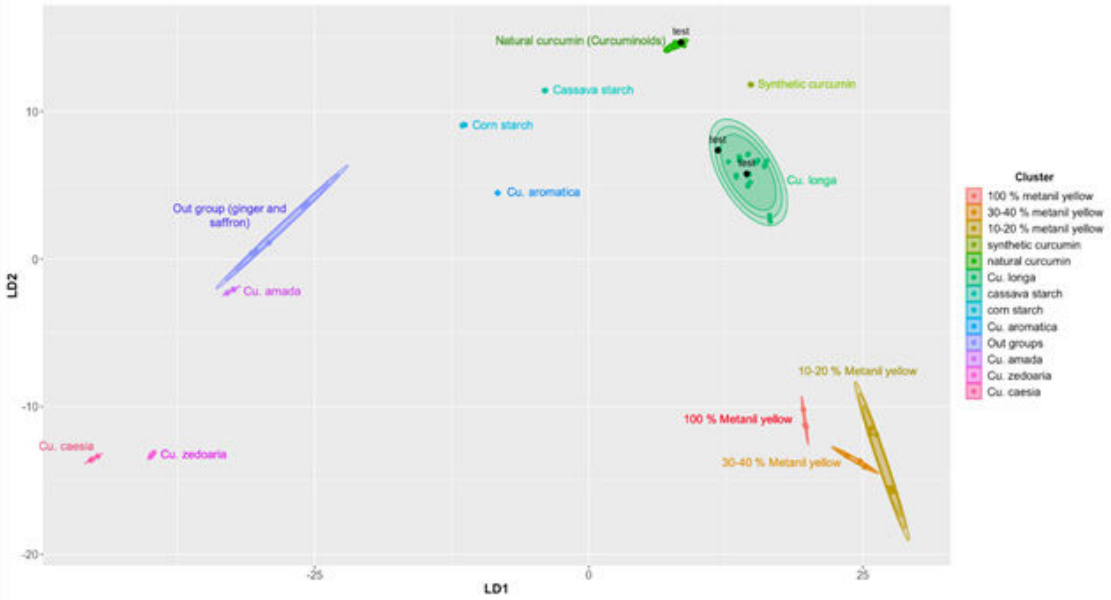

Figure 2. Multivariate statistical analysis of the NMR spectra of different plant products, metanil yellow dye, natural curcumin extract, and synthetic curcumin.

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