

Abstract

Eleven elements found in 24 cumin samples from China, India, Syria and Turkey were measured by laser ablation-inductively coupled plasma-time of flight mass spectrometry (LA-ICP-TOF-MS) for the purpose of collecting data that could be used to discriminate among the origins. Pellets were prepared of the samples and elemental concentrations obtained based on external calibration curves created using six National Institute of Standards and Technology (NIST) standards with ¹³C internal standardization. These curves were validated using NIST 1573a (tomato leaves) as a check standard. Discriminant analysis was used to successfully classify the cumin samples by their origin. Our method illustrates the feasibility of using LA-ICP-TOF-MS with an external calibration curve for high throughput screening of spice screening analysis.

Introduction

The determination of geographical origins is a very useful way to authenticate food products. In 2008, an overview of analytical techniques used to determine geographical origins of foods was published by Luykx and van Ruth [1]. Of those techniques, multi-element techniques, paired with multivariate statistical techniques have become of particular interest. The feasibility of using multi-element and multivariate techniques for the determination of origin of foods has been shown in cabbages [2,3], honey [4] and wheat [5]. Work on origin determination for spices was demonstrated by Sharp et. al, (2012) using FTIR-ATR and SIFT-MS for the discrimination and characterization of vanilla extracts [6]. Other spice work includes Brunner et al., (2010) who used ICP-MS analyze paprika samples from various countries and stages of products [7]. Most recent multi-element work on the attribution of spices involves the use of wavelength dispersive X-ray fluorescence spectroscopy (WDXRF). This work consists of using the measured elemental compositions of cumin and vanilla to differentiate sample origins using discriminant function analysis [8,9].

A method to differentiate between cumin samples originating from four different geographical regions, China, India, Syria and Turkey was developed using laser ablation inductively coupled plasma time of flight mass spectrometry (LA-ICP-TOF-MS). Discriminant function analysis of the elemental concentrations was used to differentiate the country of origin of the cumin samples and to determine the number of elements required for this determination.

Method & Materials

Samples and Standards

- Ground cumin samples obtained from McCormick & Company Inc. (Hunt Valley, MD).
- Origins included China, India, Syria and Turkey (6 each over years 2005-2010)
- Standards used for calibration curves: NIST 1515 apple leaves, NIST 1549 non-fat milk powder, NIST 1547 peach leaves, NIST 1575a pine needles, NIST 1570a spinach (Gaithersburg, MD) and GSB-30 green tea (China). (Examples shown in Figures 1 and 2).

Sample Preparation.

- 5.0 ± 0.1g cumin samples and standards were pressed into pellets (under 25 tons pressure) using a SPEX 3630 X-Press® and a 3614 40mm Evacuatable Die Set.

Method & Materials cont.

LA-ICP-TOF-MS Analysis.

- GBC OptiMass 9500 ICP-TOF-MS equipped with a cryocell set at -10C and a Cetac LSX-213 laser ablation system.
- Isotopes analyzed: ²³Na, ²⁵Mg, ³¹P, ⁴⁴Ca, ⁵⁵Mn, ⁵⁷Fe, ⁶³Cu, ⁶⁴Zn, ⁸⁵Rb, ⁸⁸Sr and ¹³⁸Ba. All isotopes were ratioed to ¹³C.
- NIST 1573a tomato leaves used as a check standard to validate the calibration curves.
 - Percent recoveries: 86.89% to 118.84% with the exception of Fe at 121.7%.
 - RSDs: 1.8% to 6.6%.

Statistical Analysis.

- Statistical analysis was carried out using Statgraphics Centurion version 16 (StatPoint Technologies Inc., Warrenton, VA, USA).
- Statistical analysis included ANOVA and discriminant analysis.

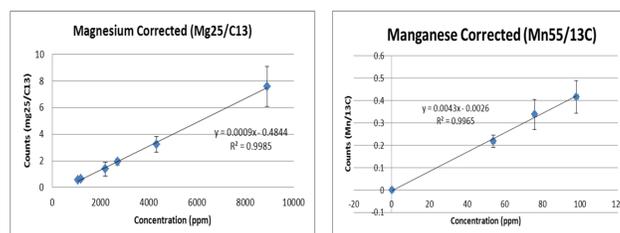


Figure 1: Example calibration curves for Mg and Mn generated using NIST standards.

Results & Discussion

The average measured elemental concentration of cumin for each country of origin is given in Table 1. ANOVA results are also given and reveal that Syria had the largest number of elemental concentrations that were statistically different from the other three countries.

Table 1- Mean ± SD for elements and their country of origin (mg*kg⁻¹) measured by the LA-ICP-TOF-MS. (n=6)

Element (mg/kg)	China Mean ± SD	India Mean ± SD	Syria Mean ± SD	Turkey Mean ± SD
Na	700.27 ± 252.06 ^a	1109.79 ± 230.30 ^b	309.19 ± 72.74 ^c	48.02 ± 14.83 ^d
Mg	1489.24 ± 350.93 ^a	1420.71 ± 223.88 ^a	1970.01 ± 301.91 ^b	970.15 ± 103.43 ^c
P	2794.82 ± 297.28 ^a	2341.77 ± 510.77 ^b	3393.11 ± 621.31 ^c	1952.24 ± 420.85 ^c
Ca	10762.40 ± 1028.41 ^a	1092.87 ± 509.91 ^a	12372.99 ± 500.05 ^b	10054.98 ± 280.23 ^c
Mn	5.40 ± 1.52 ^a	16.41 ± 6.01 ^b	13.95 ± 3.86 ^b	4.19 ± 1.00 ^a
Fe	67.20 ± 27.73 ^a	47.95 ± 22.39 ^a	136.10 ± 92.23 ^b	28.98 ± 14.09 ^a
Cu	3.13 ± 1.29 ^a	4.46 ± 1.36 ^b	6.51 ± 1.43 ^c	1.96 ± 0.28 ^d
Zn	14.01 ± 3.17 ^a	19.97 ± 6.59 ^b	32.38 ± 7.02 ^c	11.24 ± 1.38 ^a
Rb	2.07 ± 0.54 ^a	6.85 ± 3.21 ^b	5.39 ± 0.95 ^c	1.78 ± 0.62 ^a
Sr	16.51 ± 3.19 ^a	24.49 ± 8.13 ^b	20.03 ± 4.78 ^a	11.79 ± 2.93 ^c
Ba	5.31 ± 0.16 ^a	6.26 ± 0.50 ^b	7.67 ± 0.51 ^c	7.13 ± 1.01 ^d

^{abcd} Means sharing no letter in common are statistically different using LSD at 95% level.

Discriminant analysis was carried out to classify the cumin samples according to geographical origin based on their elemental compositions. 11 elemental concentrations were used as variables. Samples were separated into two categories: test samples (n=20; 5 for each of the four origins) and validation samples (n=4, 1 for each origin).

Results & Discussion cont.

3 discriminant functions were generated using a forward selection with the goal of identifying the elements that contributed most to maximizing the difference between origin clusters while minimizing the variance within each origin cluster. Of the initial 11 elements, the model required only Na, Mg, Ca, Mn, and Zn.

$$DF_1 = 1.24707*Na - 0.317234*Mg - 1.09739*Ca + 1.0946*Mn - 0.235023*Zn$$

$$DF_2 = 0.273696*Na - 1.53787*Mg + 1.1688*Ca + 0.0757567*Mn + 1.20028*Zn$$

$$DF_3 = 0.327323*Na + 1.37641*Mg - 0.413801*Ca - 0.971947*Mn + 0.0782053*Zn$$

The first discriminant function explains 77.21% of the variance in the data while df₂ explains 15.23% of the remaining variance. Figure 2 shows the resulting 2D plot of these functions. A third function is needed to describe the remaining 7.56% of the variance. The resulting 3D plot is shown in Figure 3 where each origin is clustered.

This model was tested using the validation samples. The model was able to correctly classify 100% of these samples.

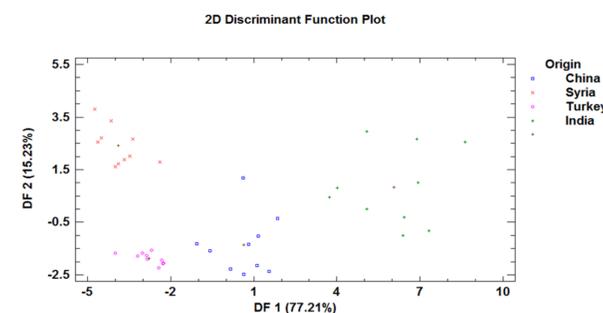


Figure 2: A 2-D discriminant function plot of cumin samples from China, Syria, Turkey and India.

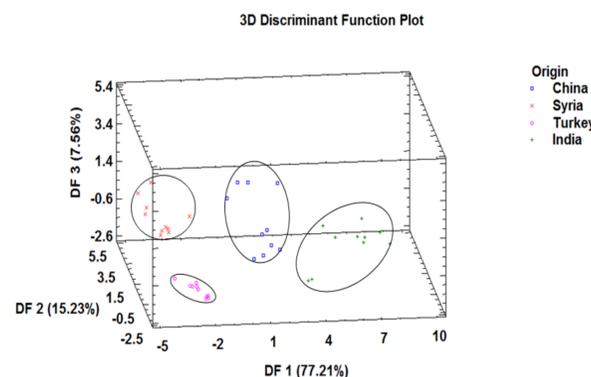


Figure 3: Three dimensional discriminant function plot for the cumin samples from four countries of origin.

Results & Discussion cont.

Method Comparison: LA-TOF vs. WDXRF

- Analysis time: LA-TOF <1 minutes vs. WDXRF ~20 minutes
- Sample Size: LA-TOF requires less sample not only for spices but for other foods.
- LA-TOF resulted in greater RSD values compared to the WDXRF.
- A complete mass spectrum obtained from each ablation using LA-TOF
- Ideal for forensic analysis and for the rapid screening of spices in accordance to the Food Safety Modernization Act.

Conclusions & Suggestions for Future

The concentrations for 11 elements found in cumin samples from China, India, Syria and Turkey were measured using the LA-ICP-TOF-MS using ¹³C correction and external calibration from NIST standards. Successful origin discrimination between the samples was achieved using discriminant functional analysis. The model was built using 3 discriminant functions, containing 5 of the 11 elements, and resulted in 100% classification of test and validation samples. The potential of the LA-ICP-TOF-MS for the rapid screening of spices and other food in accordance to the Food Safety Modernization Act has been demonstrated in this work.

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