Authenticating Spices and Herbs through DNA Barcoding

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ABSTRACT

Within the food market, there are a large number of brands offering different spices around the country, and it is unclear whether the spices we find in our local supermarkets correspond to what is advertised. Spices and herbs are a key commodity that is imported into the United States and these condiments can be expensive. It is important to understand if the biological components from different brands are indeed what the consumer is paying for. Samples of common spices from popular brands were purchased from local grocery stores in the New York Area. DNA was extracted and amplified using Ready-To-Go PCR beads. After sequencing, the samples were organized into a phylogenetic tree to confirm their identities. From the phylogenetic tree of the pilot data, it is clear the sequences for all the spices are highly correlated and have a consensus similarity of over 90% with the brand labeling.

INTRODUCTION

Spice market and Adulteration

- Spices have a high economic value because of their range of functionalities such as flavoring of food, perfumery, cosmetics, medicinal use, and preservative properties (Negi et al., 2021)
- The US dominates the spice imports of the world and is among the top 3 importers (Ly et al. 2019)
- The spice industry is under constant threat from fraud adulteration due to incorporation of low-grade, unsafe, poor-quality produce or extraneous substances. (Negi et al., 2021)
- Accidental adulteration can include insect, larvae, insect excreta, fragments, and uric acid, promoting microbial activity and spoilage properties (Negi et al., 2021)
- Food product authenticity marks a significant factor in quality and assures buyers, traders, and importing countries that the spices are of high quality and the product they intended to buy.

Emerging techniques for adulterant authentication in spices

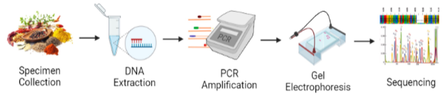
- There is an interest in confirming the correspondence of the spice alignment with its brand label both for business and customers.
- Physical methods for testing for adulteration includes macroscopic observation of texture of food materials (Negi et al., 2021)
- Chemical techniques have been widely used to determine composition of spices but are expensive and labor intensive.
- Other methods include spectroscopy, electrophoresis, and PCR based techniques

DNA Barcoding

- A short, standardized, and universal DNA sequence is read from a genetic sample.
- DNA barcoding allows us to identify samples of DNA that otherwise cannot be identified based on morphological characteristics. (Mattia, et al, 2011)
- This method allows for characterization of spice species based on miniscule plant fragments from spice products sold in powder form.

MATERIALS & METHODS

Sample collection and preparation: Spices were collected from different health and food stores in the New York area and from different commercial brands. **DNA extraction**: DNA extraction was done using Zymo Research Corporation Quick-DNA™ Miniprep Plus Kit following the suggested protocol. **DNA amplification and visualization:** DNA was amplified using Ready-To-Go PCR beads, 2µl of DNA and 23µl primer/loading dye mix. rbcLaF/rbcLa rev primers were used for amplification using the DNALC protocol. The PCR product was run on a 1.5% agarose in 1X TBE gel stained with SYBR green. Samples were sent for sequence to GENEWIZ/AZENTA Life Sciences. **Data Interpretation Plan:** DNA Subway and BLAST were utilized in order to analyze our data.



RESULTS

Common name	Brand	Reference species name
Cinnamon	La Flor - Kirkland	Cinnamomum verum
Turmeric	Swad – MDHD - Eastern	Curcuma longa L.
Cumin	Swad	Cuminum cyminum L.
Coriander	Swad	Coriandrum sativum L.
Echinacea	N/A	Echinacea angustifolia
Paprika	MDHD	Capsicum annuum
Chili Powder	Swad	Capsicum frutescens L.
Madras Curry Powder	Swad	Murraya koenigii L
Henna	Ayur - Beauty Herbal	Lawsonia inermis

Figure 1. List of analyzed spices samples divided according to their taxonomy and brand. For each sample the Reference Species name, the cultivar name or common name, for the commercial samples, are provided.

	C	1	2	3	4	5	6
Sequence Conservation							
Sequence Variation							
Consensus							
1. Curcuma_longa	C	-	91.90	96.17	98.17	98.10	99.43	99.47
2. Cinnamomum_loureiroi	1	91.90	-	88.18	89.43	89.46	91.57	90.72
3. T-F	2	96.17	88.18	-	96.37	96.17	96.17	96.74
4. S-F	3	98.17	89.43	96.37	-	97.89	98.17	97.48
5. Cinnamomum_aromaticum	4	98.10	89.46	96.17	97.89	-	97.76	99.27
6. Cinnamomum_verum	5	99.43	91.57	96.17	98.17	97.76	-	99.29
	6	99.47	90.72	96.74	97.48	99.27	99.29	-

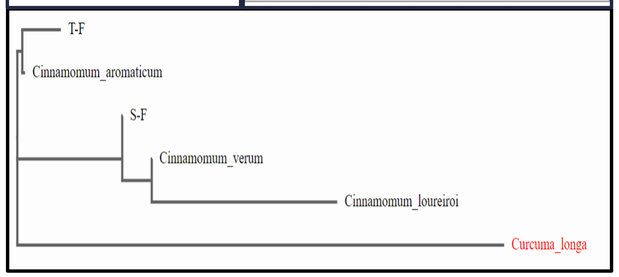


Figure 2. Sequence similarity and phylogenetic tree of La Flor (S-1) and Kirkland (T-2) Cinnamon and consensus sequences of three major species of cinnamon

Figure 3. Phylogenetic tree showing genetic relationships of Paprika MDHD data sequences
Phylogenetic tree for a maximum likelihood analysis of rbcL barcoded sequence of paprika from FASTA shows a greater genetic consensus with Bixa Orellena otherwise known as Achiote than the BLAST sequence for C*apsicum annuum*.

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1).	KP827659.1	**Zea mays cultivar Agesti 2002 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds** - Zea mays cultivar Agesti 2002 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds	599	1081	0.0	0
2(2).	HQ713391.1	**Sporobolus clandestinus ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds** - Sporobolus clandestinus ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1076	0.0	1
3(3).	Z11973.1	**Z.mays chloroplast rbcL gene for ribulose bisphosphate carboxylase** - Z.mays chloroplast rbcL gene for ribulose bisphosphate carboxylase	599	1076	0.0	1
4(4).	KM538810.1	**Coix lacryma-jobi var. ma-yuen ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds** - Coix lacryma-jobi var. ma-yuen ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1072	0.0	2

Figure 4. BLAST results for Maca, *Lepidium meyenii,* from a Peruvian open market shows that the main component of the mix is corn.

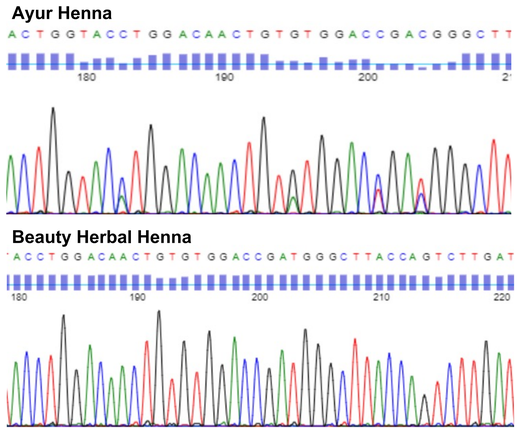


Figure 5. Sequence viewer of Ayur Henna and Beauty Herbal Henna
Peaks in the electropherogram correlate to nucleotide positions in the DNA sequence. Software program Phred examines the peaks around each call and assigns a quality score for each nucleotide. All the bars have a Phred score higher than 20 and are characterized by distinct peaks indicating high quality sequences. Several overlaps are recurrent throughout the sequence viewer for Ayur Henna indicating a discrepancy in nucleotide recognition. We propose this is because other substances were added to the *Lawsonia inermis* (henna) by the vendor. As a comparison sample Beauty Herbal Henna showed no overlaps or discrepancies throughout the sequence viewer.

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1).	MG946829.1	**Coriandrum sativum ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds** - Coriandrum sativum ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds	599	1076	0.0	1
2(2).	LC633823.1	**Coriandrum sativum Chiang Mai University, Faculty of Pharmacy:COS-CM11032021 chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds** - Coriandrum sativum Chiang Mai University, Faculty of Pharmacy:COS-CM11032021 chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	601	1072	0.0	1
3(3).	KT178115.1	**Cicuta maculata voucher Kellar 1390 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds** - Cicuta maculata voucher Kellar 1390 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds	599	1063	0.0	4

Figure 6. BLAST results for Curry Powder have high genetic consensus with Coriandrum sativum and *Cicuta maculata*, a toxic plant found in North America in addition to a few other plants. The toxic ones are the ones that piqued our interest, and we plan to repeat the test. However, it is also be a clue to the limitations of the testing, particularly when using only a maker for a complex samples.

DISCUSSION

Spices from our pilot data samples match closely with brand labeling

- Our data indicate that vendors are generally accurately branding their products. In Figure 2, both brands of cinnamon share over a 91% consensus similarity with the three most common species of cinnamon sold on the market.
- We did find that at least one of our paprika samples contained achiote in stead of the expected spice, Figure 3.
- We were also able to prove that the maca herb, *Lepidium meyenii* bought on a market in Peru contained in fact mostly corn, Figure 4.
- We also observed with the henna samples how the gene selected can help distinguish between different species, but also with the Curry sample the possible limitations of the system, Figure 5 and 6.

Future research will investigate more expensive spices and herbs and use more than one marker when using more complex samples

- We would like to test more brands of Paprika to see if it is common for achiote to be used as a substitute.
- We would like to look at saffron as harvesting saffron is very labor intensive and authentic saffron can be sold from $1600 t0 $5000 per pound
- Other samples we were considering were to test maca from a health store in the US, vanilla and cardamom.
- As these spices have high monetary value there is a better chance for them to be adulterated.

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ACKNOWLEDGEMENTS

We would like to express our deepest gratitude for the following people for helping me successfully complete this project:

Dr. Raffaella Diotti for her utmost dedication and support in furthering our research experience through feedback, patience, and enthusiastic encouragement throughout the past year. Dr. Diotti's insightful feedback pushed us to sharpen our thinking and brought our work to a higher level.

Dr. Jeremy Seto for allowing us to work in his lab for the fall and for his dedication and useful critiques of this research work. Dr. Seto's expertise and mentorship was invaluable in formulating the methodology of this project.

Dr. Mayle, Dr. Hackett, and Arden for their advice and assistance in keeping our progress on schedule. We want to thank you for your patient support and for all of the opportunities we were given to further our research.