^{1}H NMR-BASED METABOLOMICS AS A POWERFUL TOOL FOR THE AUTHENTICATION OF THE "KING OF SPICES": BLACK PEPPER (*PIPER NIGRUM* L.)

IX SIMPOSIO de Investigación EN CIENCIAS EXPERIMENTALES

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1. INTRODUCTION

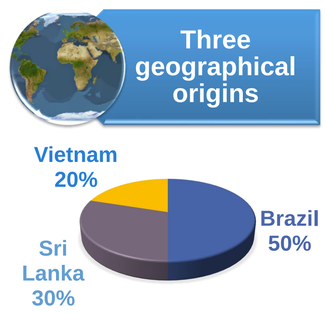
Nowadays, there is a growing demand of natural products such as spices due to the consumer belief that "the natural is the healthiest". Black pepper (*Piper nigrum* L.), also known as the "king of spices" or "black gold", is a plant belonging to the Piperaceae family and it is largely used as a condiment in foods due to its commercial, nutritional, and medicinal value. It is not only the most consumed spice in the world, but also the most traded, being Vietnam, Brazil, and Sri Lanka one of the largest producers [1]. A huge variety of naturally occurring metabolites has been found in black pepper, being the alkaloid piperine the main bioactive constituent which is also responsible for the characteristic pungency and biting taste of this highly valued spice [2].

Proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR) is well-suited to the analysis of complex samples because it is reproducible and the sample preparation is not time-consuming. Additionally, it can identify variations in the metabolomic fingerprint of a certain plant so that, once ^{1}H NMR fingerprints are recorded, chemometrics can be subsequently applied to reduce the complexity of the data to detect the pattern of changes related to environmentally or genetically induced variations in metabolite composition.

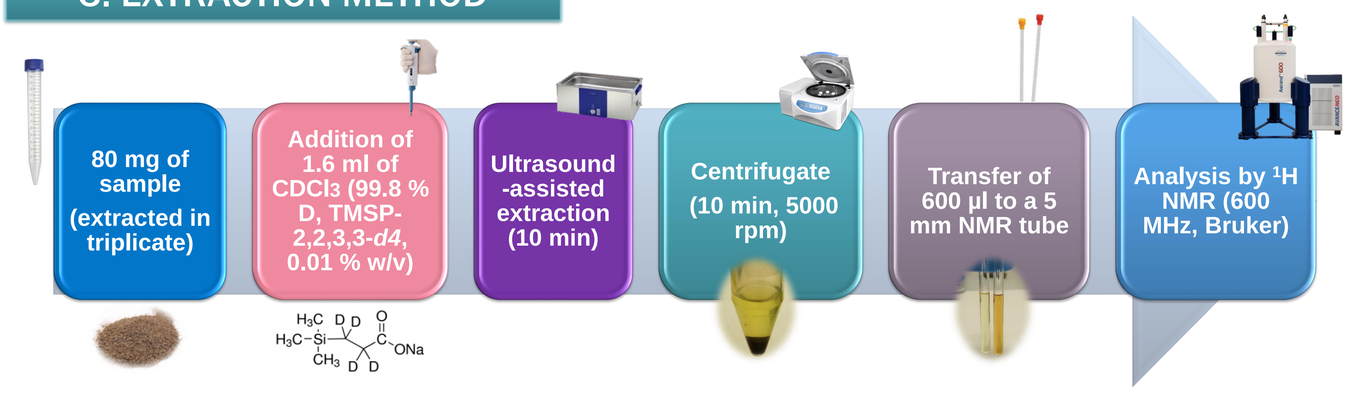
In this study, ^{1}H NMR spectroscopy together with metabolomic tools was used for the first time for the successful authentication and classification of 20 samples of black pepper from three geographical origins (Brazil, Vietnam, and Sri Lanka), and two quality processing (sterilized and non-sterilized spice) based on their corresponding ^{1}H NMR fingerprints.

2. STUDIED SAMPLES

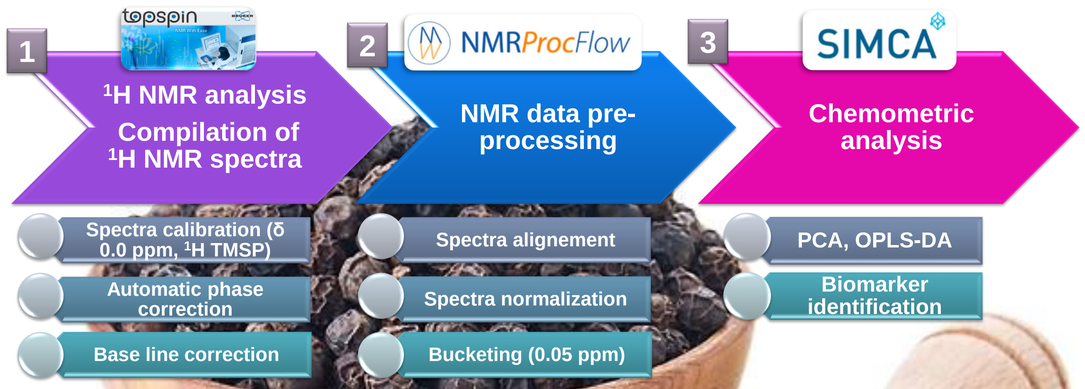
20 SAMPLES OF BLACK PEPPER (*Piper nigrum* L.)
(Sabater Spices, Murcia, Spain)



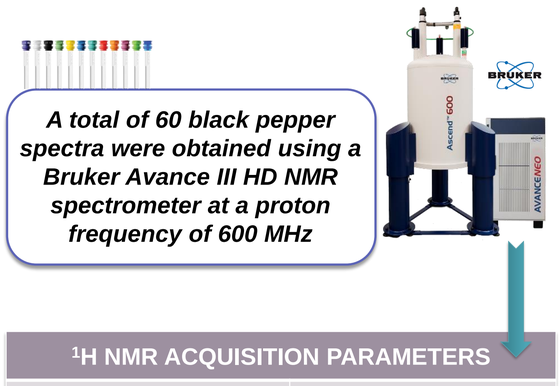
3. EXTRACTION METHOD



4. UNTARGETED METABOLOMIC WORKFLOW



5. ^{1}H NMR ANALYSIS



A total of 60 black pepper spectra were obtained using a Bruker Avance III HD NMR spectrometer at a proton frequency of 600 MHz

^{1}H NMR ACQUISITION PARAMETERS	
Nucleus	^{1}H
Proton frequency	600.13 MHz
Optimized solvent	$CDCl_3$
Internal standard (IS)	TMSP-2,2,3,3-*d4*
Bruker pulse sequency	zg30
Number of scans	32
Spectral width	14 ppm (8417 Hz)
Transmitter frequency	5 ppm (3001 Hz)
Relaxation delay (D1)	1 sec
FID size (TD)	65536
Receiver gain	18
Acquisition time (AQ)	3.89 sec

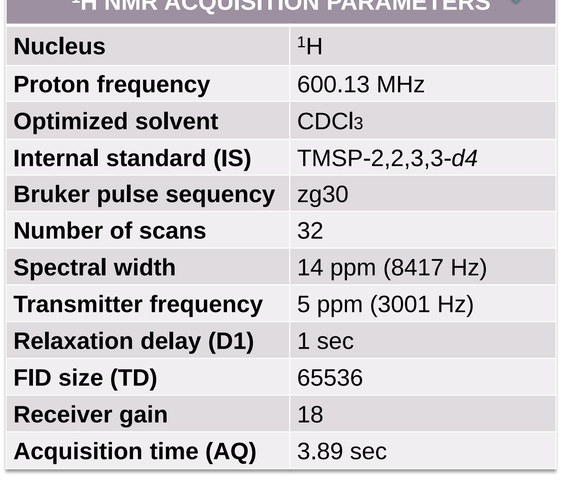


Figure. ***Overlapped ^{1}H NMR spectra of the investigated black pepper samples (600.13 MHz, $CDCl_3$)***

6. CHEMOMETRIC ANALYSIS. RESULTS AND DISCUSSION

NON-SUPERVISED ANALYSIS

1 PRINCIPAL COMPONENT ANALYSIS (PCA)

It shows the tendency of black pepper samples to <u>form clusters based on their metabolite profiles</u> according to their geographical origins and quality processing

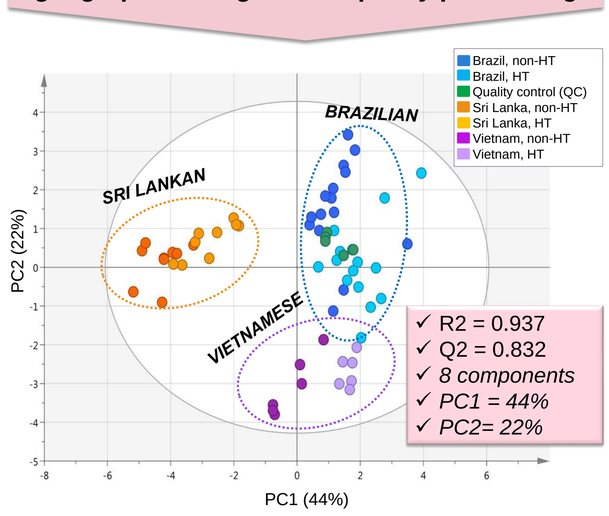


Figure. ***PCA score plot according to origins and quality processing of the black pepper samples***

SUPERVISED ANALYSIS

2 ORTHOGONAL PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS (OPLS-DA)

Reliable and excellent <u>discrimination of black pepper samples</u> according to their geographical origins as well as their quality processing by ^{1}H NMR spectroscopy combined with chemometrics

GEOGRAPHICAL ORIGIN DISCRIMINATION

100% samples correctly classified

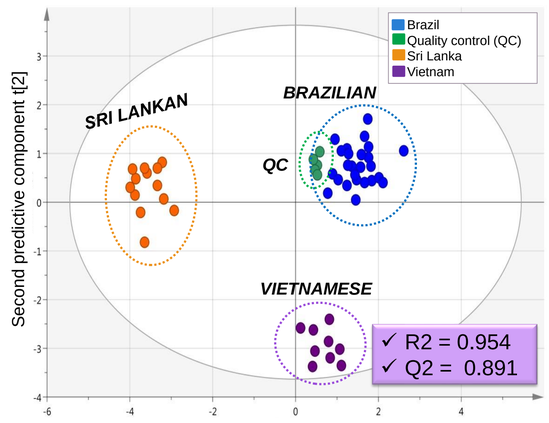


Figure. ***OPLS-DA score plot according to the geograhical origins***

QUALITY PROCESSING DISCRIMINATION

92% samples correctly classified

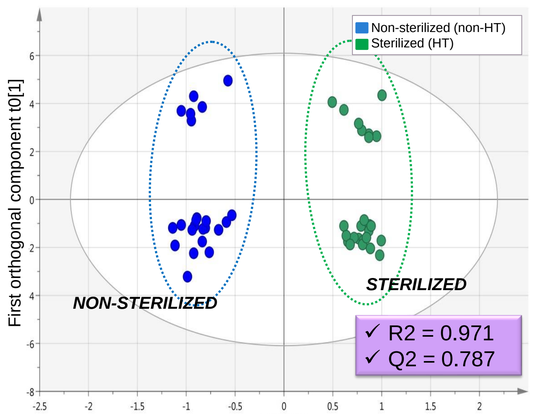


Figure. ***OPLS-DA score plot according to the quality processing***

IDENTIFICATION OF BIOMARKERS

Variable Importance in Projection (VIP) plot

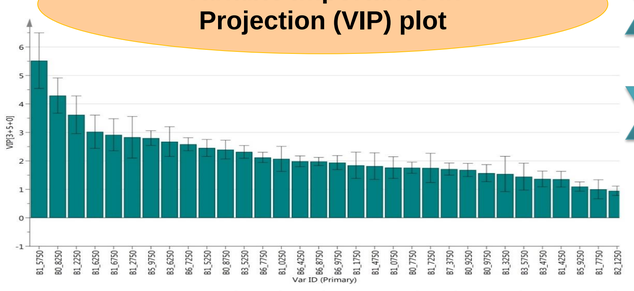


Figure. ***VIP score plot showing NMR buckets of potential biomarkers***

It provided a list of variables (NMR buckets) that are responsible for the discrimination of the studied samples

Most of the variables having the highest discrimination potential (VIP values > 1.0) correspond to

THE ALKALOID PIPERINE

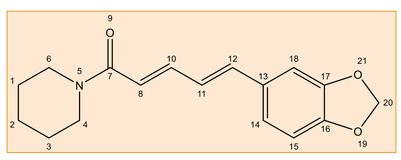


Figure. ***Chemical structure of piperine***

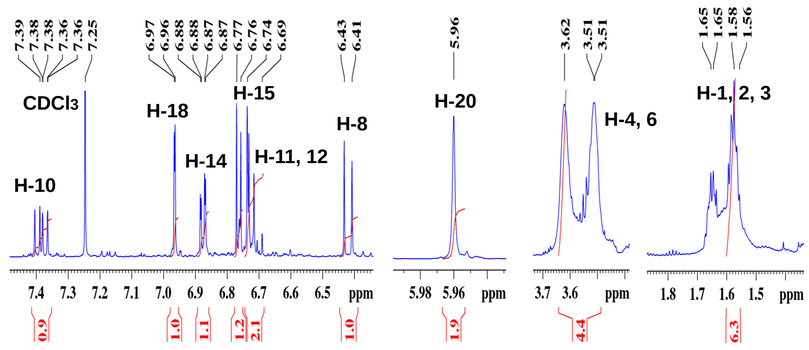


Figure. ***Piperine identification in ^{1}H NMR spectra (600.13 MHz, $CDCl_3$)***

7. CONCLUSIONS

- ✓ **This research studied for the first time the metabolomic profile of the spice black pepper using NMR spectroscopy combined with metabolomic tools**
- ✓ **A simple ultrasound-assisted extraction (UAE) method have been optimized for the obtention of the sample fingerprints and it was a suitable extraction method for the further direct analysis by ^{1}H NMR**
- ✓ **PCA was used as an exploratory tool to overview the tendency of the samples to form clusters. A tight QC cluster was observed in the score plots indicating a good robustness and reproducibility of the chemometric results**
- ✓ **The proposed OPLS-DA models allowed the correct discrimination and classification of a high portion of the samples (92-100%) according to their geographical origins (Brazil, Vietnam, and Sri Lanka) and the quality processing they have been subjected to (sterilized or non-sterilized)**
- ✓ **Piperine was identified in $CDCl_3$ black pepper extracts and it was pointed out as the biomarker having the highest discrimination potential**

8. ACKNOWLEDGMENTS

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9. REFERENCES

[1] J.L. Lafeuille, A. Frégière-Salomon, A. Michelet, K.L. Henry. J. Agr. Food Chem. 2020, 68, 390–401.

[2] A.B. Sharangi, *Indian Spices: The legacy, production and processing of India's treasured export*, Springer, 2018.