IDENTIFICATION OF BIOMARKERS AND CHEMICAL DESCRIPTORS OF TASTE AND AROMA IN NATIVE PERUVIAN CHILI PEPPERS THROUGH A METABOLOMIC APPROACH

Fabio Espichan[1], Guillaume Marti[2], Fredy Quispe[3], Rosario Rojas[1],Edgard Asencios[1]

[1]Universidad Peruana Cayetano Heredia. [2]Faculté de Pharmacie-Université Paul Sabatier-Toulouse III, 35 chemin des Maraîchers, 31400 Toulouse-Francia. [3]Instituto Nacional de Innovación Agraria (INIA)

ABSTRACT

The genus *Capsicum* involve 30 species, including 5 that have been domesticated: *Capsicum annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*. Although the genus *Capsicum* has been studied from a taxonomic point of view, there are still some problems related to the taxonomic delimitation of the genus and its species. *C. annuum*, *C. chinense* and *C. frutescens* form a taxonomic complex whose discrimination between species and varieties is still difficult to carry out. The flavor and aroma are attractive attributes of importance to the consumers of chili peppers, the knowledge of their attributes will allow the improvement of the quality. In the present work we report the metabolomic study of 5 varieties of Peruvian native peppers: "Ayucllo" and "Red Tomatito" (*C. baccatum*); "Miscucho" and "Dulce rojo" (*C. chinense*) and "Charapita amarillo" (*C. frutescens*). The agronomic research was carried out in three localities of Peru: Chiclayo, Chincha and Huaral. Two datasets were obtained by two analytical platforms (GCMS, UHPLC-HRMS) along a sensory dataset. The sensory analysis was carried out by a trained tasting panel constituted by 9 tasters using ten descriptors of flavor and aroma. The acquisition of non targeted metabolomic data by GCMS was carried out by solid phase microextraction (SPME) and ionization by electronic impact (EI). UHPLC-HRMS was performed in two ionization modes (ESI (+) and (-)) on reversed phase column. The multivariate analysis by Principal components (PCA) clustered samples according to their species for both GC and LC-MS approaches. Discriminant analysis by OPLS-DA (Orthogonal projection to latent structure-discriminant analysis) highlighted characteristic biomarkers of each species. A Multiple Factorial Analysis (MFA) was used to filter the most well-modeled sensory descriptors in consensus with the perceptions of the panelists and to correlate the tendencies between flavor and aroma descriptors linked to the three species. To correlate the GCMS, UHPLC-HRMS, and sensory datasets a Multi-Block Holistic Integration Analysis was set up using the DIABLO approach. Our results displayed the common or correlated information among the three datasets, optimally identifying key omic variables, which explained and classified reliably subgroups or phenotypes of interest. The validation of the model was carried out through cross-validation (p-value = 0.001), using the Mahalanobis distance classification criterion.

EXPERIMENTAL PROCEDURE

CAPSICUM SAMPLES

The samples of *Capsicum* were provided by the National Institute of Agrarian Innovation (INIA) from its 2 experimental stations located in Chiclayo and Huaral and by the AgroExport Topara company in Chincha Peru. They cultivated 5 accessions of peppers, their common names are: Ayucllo, Miscucho, Red Tomatito, Charapita and Red Sweet (Figures 1-5).

Figura 1. Charapita amarillo (*Capsicum frutescens*)

Figura 2. Ayucllo (*Capsicum baccatum*)

Figura 3. Miscucho (*Capsicum chinense*)

Figura 4. Dulce rojo (*Capsicum chinense*)

Figura 5. Tomatito rojo (*Capsicum baccatum*)

SAMPLE PREPARATION, LYOPHILIZATION

Fresh chilli samples were frozen at -19 °C and then dried by lyophilization at a pressure of 0.5 mbar and a temperature of -40 °C for 2 days. The lyophilization yield was approximately 10%. Finally, the samples were ground to a fine powder (Figure 6).

Figure 6. Lyophilized and ground samples of *Capsicum*

MATERIALS AND METHODS

Ultra high efficiency liquid chromatograph (UHPLC) coupled to high resolution mass spectrometer (HRMS) with Orbitrap LTQ-XL EDT analyzer and UV, PDA and CAD detector of the Thermo brand with resolution 60,000 to 400m / z and electrospray ionization (ESI) modes (+) and (-) was used for the metabolomic studies.

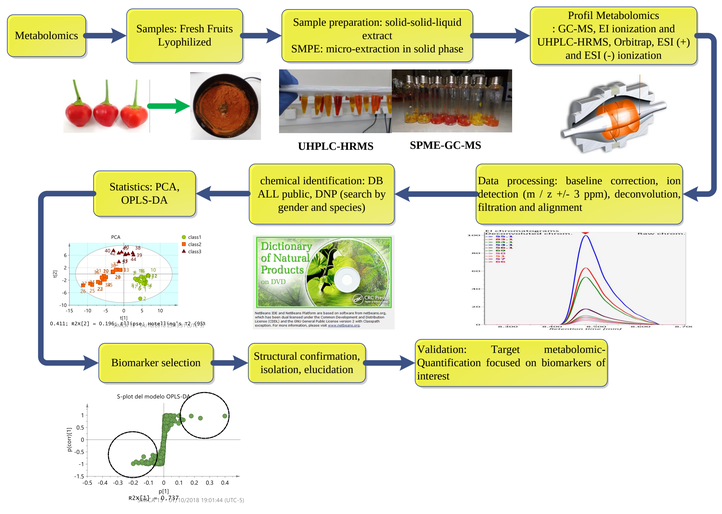
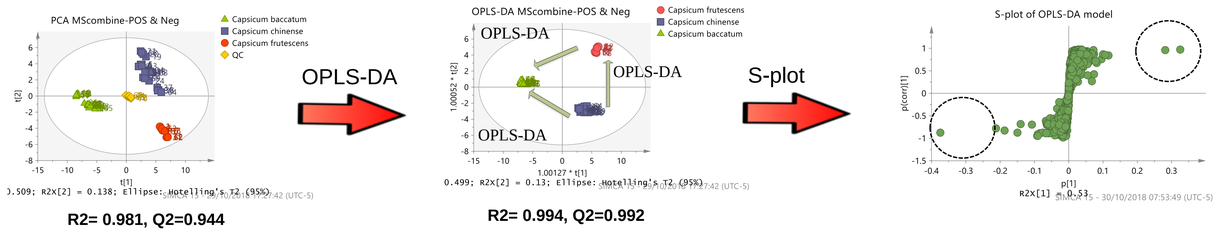


Figure 7. Metabolic workflow for the identification of biomarkers

MULTIVARIATE ANALYSIS (MVA)

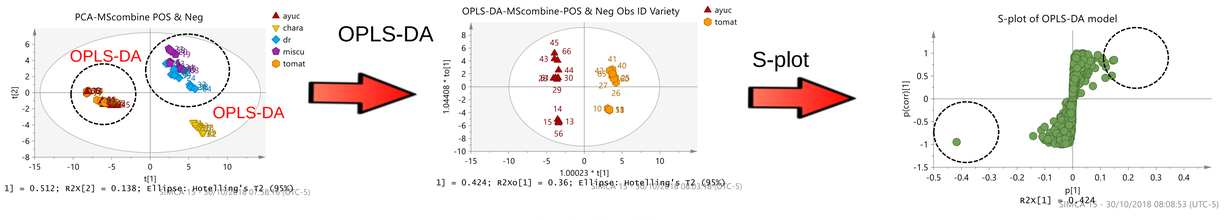
MVA FOR SPECIES



R2= 0.981, Q2=0.944

R2= 0.994, Q2=0.992

MVA FOR VARIETY



R2= 0.986, Q2=0.954

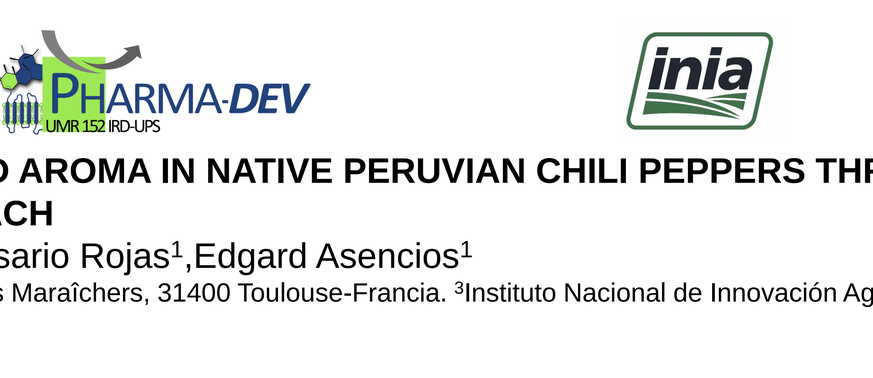
R2= 1, Q2=0.992

Ayuc = ayucllo
Chara= charapita
dr= Dulce rojo
Miscu = Miscucho
Tomat= Tomatito rojo

Tabla 1. Main metabolites biomarkers for the discrimination between Varieties and Species of *Capsicum*

Species	Accurate mass	Molecular formula	Family compound	Biomarkers	Found in:	Total Score
C. baccatum	418.17802	C26H26O5	Flavonols; three O substituents	Macaflavone I; 3'-Deoxy, 4'-Me ether	DNP, Solanaceae	7,4392
C. chinense	279.18344	C16H25NO3	Lycopodium alkaloids	Serratezomine A	DNP, Solanaceae	7,4193
C. frutescens	364.22497	C21H32O5	Monocarbocyclic aldehydes and ketones	2-Hydroxy-12-oxo-10,15-phytodienoic acid; Ac, Me ester	DNP, Solanaceae	7,9005
Variety	Accurate mass	Molecular formula	Family compound	Biomarkers	Found in:	Total Score
Ayucllo	432.2148	C24H32O7	Cembrane diterpenoids	6,10,14-Trihydroxy-3,7,11,15(17)-cembratetraen-16,2-olide; 10,14-Di-Ac	DNP, Solanaceae	7,4796
Tomatito rojo	384.10565	C17H20O10	Benzopyranoids - 6,7-Dioxygenated coumarins	6,7-Dihydroxy-2H-1-benzopyran-2-one; 7-O-[4-O-(2-Hydroxyethyl)-b-D-glucopyranoside]	DNP, Solanaceae	7,2864
Miscucho	530.16356	C23H30O14	Benzopyrans	5,6,7-Trihydroxy-2-methyl-4H-1-benzopyran-4-one; 6-Me ether, 7-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside]	DNP, Capsicum	7,3593
Dulce rojo	394.14751	C16H26O11	Iridoid monoterpenoids	Scutelloside; 6-Me ether	DNP, Capsicum	7,3374

MULTIPLE FACTORIAL ANALYSIS-AFM



MULTIBLOCK ANALYSIS

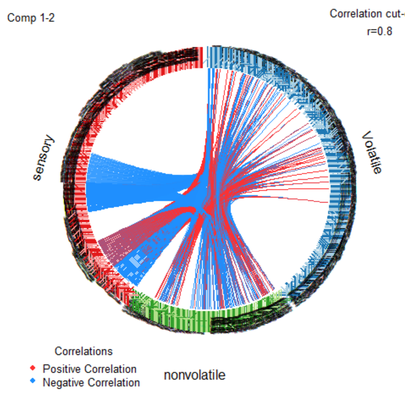


Figure 8. CircosPlot of Correlations

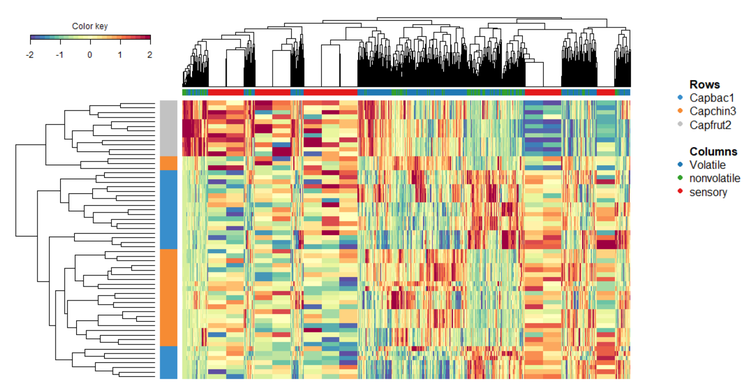


Figure 9. Heatmap of Correlations

Table 2. Compounds with significant contribution in the attribute prediction

Attribute	Descriptors	Metabolite	Taste profile	Flavor profile	Data base	Corr
Odor	Oregano	Pyrazine, 2-methoxy-3-(2-methylpropyl)-	NA	spice, pepper	FlavorDB	-0.339
	Herb	Hexanoic acid, 3-hexenyl ester	NA	grassy, green	FlavorDB	-0.532
	Apple	Hexyl butyrate	NA	apple	FlavorDB	-0.329
	Citric	6-Octen-1-ol, 3,7-dimethyl- (Citronellol)	NA	citrus	FlavorDB	0.39
	Fruity	Cyclobutanecarboxylic acid, hexyl ester	NA	fruity	FlavorDB	0.766
	Passion fruit	Hexyl butyrate	NA	Passion fruit	FlavorDB	-0.227
Taste	Acidity	Daucic acid	Red beetroot	NA	FOODDB	-0.511
	Sweet	Vulgaxanthin II	Red beetroot	NA	FOODDB	-0.847
	Red pepper	Isofraxidin	Muskmelon, Watermelon	NA	FOODDB	0.811
	Tomato	Benzoic acid	Garden tomato	NA	FOODDB	-0.731

FlavorDB: http://foodb.ca/
FOODDB: http://cosylab.iiitd.edu.in/flavordb/search

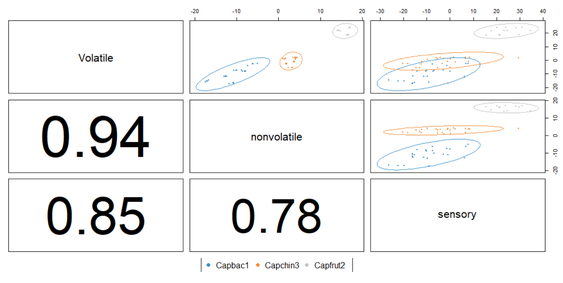


Figure 10. Criterion of inter-block correlations

CONCLUSIONS

The metabolomic approach is a tool that has allowed us to make the chemical differentiation between varieties and species. It has been possible to identify the biomarkers that allow differentiation between varieties ayucllo and red tomato (*C. baccatum*), as well as between Miscucho and red sweet (*C. chinense*).

The multiblock analysis is a multivariate tool that has allowed us to correlate the GCMS, UHPLC and sensory data tables and find the chemical descriptors of the flavor and aroma of the 5 varieties of Peruvian chili peppers.

BIBLIOGRAPHY

1. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). Journal of Chemometrics. 2002;16(3):119-28.
2. Wiklund S, Johansson E, Sjöström L, Mellerowicz EJ, Edlund U, Shockcor JP, et al. Visualization of GC/TOF-MS-Based Metabolomics Data for Identification of Biochemically Interesting Compounds Using OPLS Class Models. Analytical Chemistry. 2008;80(1):115-22.
3. Bylesjö M, Rantalainen M, Cloarec O, Nicholson JK, Holmes E, Trygg J. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. Journal of Chemometrics. 2006;20(8-10):341-51.
4. Eggink PM, Maliepaard C, Tikunov Y, Haanstra JPW, Bovy AG, Visser RGF. A taste of sweet pepper: Volatile and non-volatile chemical composition of fresh sweet pepper (*Capsicum annuum*) in relation to sensory evaluation of taste. Food Chemistry. 2012;132(1):301-10.

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