

FLAVOROMICS APPROACH TO DESCRIBE THE SENSORY PROPERTIES OF A CUP OF COFFEE

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AIM AND SCOPE

Flavoromics is an “-omic” and “-holistic” approach focused on low molecular mass compounds (volatile and non-volatile) and linking them to a defined sensorial perception, thanks to advanced chemometric techniques [1-3]. Flavor perception is a multi-modal evaluation as the result of the interaction between different and complex chemical stimuli (ortho retro-nasal, taste, texture, etc.) and the (bio)chemical and physiological responses. Panels are frequently time and resource intensive, so cost and time effective complements should be developed. Correlation between instrumental analysis and panel perception is made difficult by the complexity of the coffee flavor and different study has attempted to associate them [4-5]. This work deals with the study of a flavoromics approach to describe and predict the bitter sensory note of coffee; its potential and limits in a routine control purpose is also discussed. Chemical information from coffee samples was obtained by analyzing both volatile and non-volatile profiles, followed by processing of the resulting chromatograms to reduce the number of variables with statistical tools. Multiple Factorial Analysis was used to study the relationship between the observations, (volatiles, non-volatiles, and sensory scores) and Partial Least Squares Regression analysis to correlate chemical data with well-defined flavor notes in a cup of coffee determined by monadic sensory analysis. Some variables were selected as diagnostic markers, because of their high impact on the statistical models developed. Bitter flavor markers different from caffeine were highlighted as well [6-7]. Targeted analysis on these compounds is in progress.

Materials and Methods

Samples

Coffees samples, consisting of roasted coffee ground to suit a coffee-filter machine, were kindly supplied over a period of 12 months by Lavazza Spa (Turin, Italy). Fifty coffee samples with distinctive sensory notes originating from different countries belonging to the species *Coffea Arabica* L. (Arabica) and *Coffea canephora* Pierre (Robusta) were analyzed. The roasting degree of each sample was carefully measured by ground bean light reflectance, with a single-beam Neuhaus Neotec Color Test II instrument (Gendekesee, Germany) at a wavelength of 900 nm on 25-30g of ground coffee. Roasting degree was set at 55°N_h, in order to be close to the international standardization protocol for cupping. Samples were roasted within 24 hours prior to cupping, and left for at least 8 hours to stabilize.

HS-SPME-GC-MS Analysis of the coffee powder

HS-SPME sampling was carried out with a QP2010 GC-MS system equipped with an autosampler combi-PAL AOC 5000 Autoinjector (Shimadzu - Milan, Italy). 1.5 g of powder were weighed in a septum-sealed glass vial (20mL); the resulting headspace was sampled through the SPME fiber for 40 minutes at 50°C with an agitation speed of 350rpm. Tridecane (C13) in Dibutylphthalate (DBP), was used as internal standard, were purchased from Sigma-Aldrich (Milan-Italy). The internal standard was pre-loaded onto the fiber (Wang, O'Reilly, Chen, & Pawliszyn, 2005) in advance by sampling 5µL of a 1000mg/L solution of n-C13 in DBP into a 20mL headspace vial for 20 min at 50°C, agitation speed of 350rpm. Each sample was analyzed in two technical replicates.

Analysis Conditions. injector temperature: 230°C; injection mode, splitless; carrier gas, helium (2mL/min); fiber desorption time and reconditioning, 5min; column, SGE SolGelwax (100% polyethylene glycol) 30 m x 0.25 mm dc x 0.25 µm df (SGE- Melbourne, Australia); temperature program, from 40°C (1min) to 200°C at 3°C/min, then to 250°C (5min) at 10°C/min. MS conditions: ionization mode: EI (70eV); scan range: 35-350 amu; ion source temperature: 200°C; transfer line temperature: 250°C. Data were collected with a Shimadzu GCMS Solution 2.5SU1. Aroma compounds were identified through their linear retention indices and EI-MS spectra, compared to those of authentic standards or, tentatively, to those collected in-house or commercial libraries (Wiley 7N and Nist 05 ver 2.0 Mass Spectral Data).

Preparation of Coffee Extracts

The coffee brew for cupping and analysis was prepared from 18g of coffee powder and 300mL of water, using a commercially available “Xlong” coffee filter machine. Two milliliters of brew are then filtered on a 0.2 µm 13 mm nylon membrane syringe filters (Agilent, Little Falls, DE, USA) and 20 µL directly injected.

HPLC-UV/DAD Analysis

The HPLC-UV/DAD analyses were carried out on a Agilent 1200 system provided with a Spectra System UV Diode Array Detector 1100 series (Agilent, Little Falls, DE, USA). Data acquisition and data handling were performed with Chemstation LC 3D system software Rev 3.03 01-SR1 (Agilent, Little Falls, USA). HPLC column was a Platinum EPS C18 (250x4.6 mm, 80Å, 4 µm) (Alltech, Deerfield, USA). Operative conditions were: injection volume, 20 µL; detection wavelength, 325 nm for cinnamic and chlorogenic (monomers and dimers derivatives) and 276 nm for caffeine and trigonelline; mobile phase, ACN/H₂O-ACN 0.1% formic acid; flow rate, 1.0 mL/min; mobile phase program, from 85% H₂O (7 min) to 45% H₂O (20 min) to 100% ACN hold for 2 min. Before re-injection, the HPLC system was stabilized for at least 5 min.

Data processing

The targeted GC and untargeted HPLC-UV/DAD fingerprints were used to extract information about samples. Multiple Factorial Analysis (MFA) was used to define relationship between the non-volatile and volatile fraction to describe samples characterized by different aroma. Data reduction to define aroma and taste features most related to the bitter note was made through a partial least square-discriminant analysis (PLS-DA). Partial least square (PLS) regression was performed on both set of data (GC and HPLC-UV/DAD) separately and then fused into a single data set to determine the prediction ability in defining the bitter sensory scores and results compared to those obtained from the panel. MFA was run by XLSTAT (version 2015.5.01.23164) software, copyright Addinsoft 1995-2015. PLS-DA and OPLS (Orthogonal Partial Least Square analysis) was made with Pirouette ver 4.0 rev 2 (Infometrix, Wa, USA).

RESULTS AND DISCUSSION

Multiple Factor Analysis (MFA) makes it possible to analyze the three data matrix/tables (targeted fingerprints from GC, untargeted from HPLC-UV/DAD and descriptive sensory analysis) simultaneously, and provides results capable of assessing the relationship between the observations, the variables and tables. Results, expressed as RV coefficients, indicate to what extent the tables/variables distribution are related two by two. The more the variables from the GC evaluation (aroma) are related to the variables of the HPLC-UV/DAD (taste) or the sensory scores for the attributes investigated, the higher the RV coefficient (variation range 0-1). In this case, the mutual correlation between the aroma and taste data set was 0.920 indicating a great accordance between their chemical fingerprint informative content (aroma and taste), while the sensory analysis matrix results relatively less correlated compared to chemical data. The originality of method is that it affords to visualize in a two or three dimensional space, the tables (each table being represented by a point), the variables, the principal axes of the analyses and the individuals. In addition, the method can evaluate the impact of the other tables on an observation by visualizing simultaneously i) the observation described by all variables and ii) the projected observations described by the variables of only one table. Graphical results of Figure 1 displays the coordinates of the points projected in the MFA space.

	Aroma	Taste	Sensory	AFM
Aroma	1.0000	0.9206	0.5493	0.9514
Taste	0.9206	1.0000	0.5053	0.9338
Sensory	0.5493	0.5053	1.0000	0.7479
AFM	0.9514	0.9338	0.7479	1.0000

Table 1 RV Coefficients of relationship between the tables. The value of the RV coefficients varies between 0 and 1.

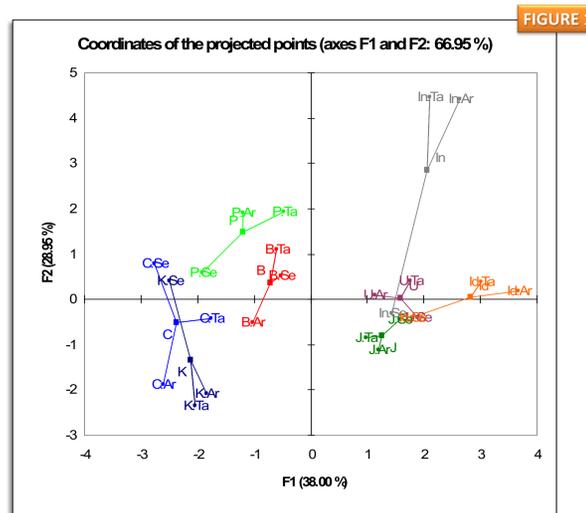


Figure 1 Coordinates of the projected points in the space resulting from the MFA. Ar: aroma, Ta: taste, Se: sensory. C: Colombia, P: Papua Nuova Guinea, In: India, U: Uganda, Id: Indonesia, B: Brazil, H: Kafa, J: Java.

FIGURE 2

Model parameters in validation and prediction	External Test Set		Bitter evaluation on External Test Set	
	Bitter	samples	meas	pred
SEP ^b	1.60	37	0.33	0.80
PRESS	20.37	40	2.90	2.52
r pred ^a	0.64	53	0.83	1.52
Factors	1.00	75	0.18	1.40
Slope	0.44	79	3.30	3.84
Intercept	0.99	114	0.00	-1.02
r val ^c	0.85	BUK	6.47	2.65
SEV ^d	1.02	INDOAP	1.65	3.11

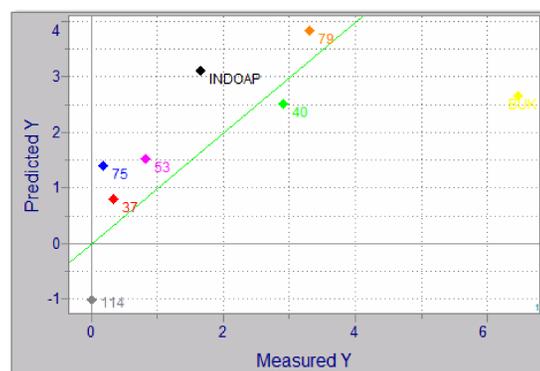


Figure 2 OPLS regression model for bitter scores prediction as an association measure between volatiles fingerprint and sensory scores of 8 coffee samples. ^aCorrelation coefficient in prediction, ^bStandard error in Prediction, ^cCorrelation coefficient in Validation, ^dStandard error in Validation. Autoscaling of raw normalized data.

The projected points correspond to projections of the observations in the spaces reduced to the dimensions of each table. The representation of the projected points superimposed to those of the complete observations makes it possible to visualize at the same time i) the diversity of the information brought by the three data set corresponding to the samples, and ii) to visualize the relative distances. As shown, aroma and taste data matrix are closely correlated, and the relative position of the observations on the Cartesian plane coherently distributed.

Using aroma targeted fingerprint to characterize the bitter note might successfully be used instead of taste untargeted fingerprint since they appear to provide the same chemical information. To do this, the most correlated variables with the bitter sensory attribute were extracted with PLS-DA and once selected, used to perform an OPLS regression in order to predict the sensory score for bitter note. The OPLS regression model on the first 3 PCs was first calculated and internally cross validated (3 CV) on the training data set from the aroma fraction and then applied to an external test set of samples.

Figure 2 shows the model parameters together with its ability in scores prediction. Aroma compounds most related to the model prediction were pyrazines (2-isopropenylpyrazine, 2-n-propylpyrazine, 1-methylethyl pyrazine, 2,3-dimethylpyrazine, 2-methyl-6-(1-propenyl) pyrazine, 3,5-diethyl-2-methyl pyrazine, 3-ethyl-2,5-dimethyl pyrazine), and guaiacol derivatives (Guaiacol, 4-ethyl Guaiacol, 4-vinyl Guaiacol) and phenethyl alcohol.

The PLS regression model relative to taste (data not shown) displays good results in determining the sensory scores (r² in prediction is 0.965 with a prediction error of 0.58) showing that, as expected, this fraction of taste better describe the bitter sensory note. Bitter related variables showed other chemicals contributing to the bitter note other than caffeine. Among them, some major compounds belonging feruloylquinic acids and di-caffeoylquinic derivatives together with several other minor components result also strongly related to the bitter note [6-7]. Targeted analysis by HPLC-DAD-ESI/MSn to identify them is in progress.

However, in coffee cupping, both aroma and taste are considered (orto-nasal impression, all gustatory (taste bud) sensations and retro-nasal aromas). Moreover, the evaluation intended to define different attributes at the same time. This multimodal perception could be represented when using an aroma and taste fused data approach. Results are reported in figure 3 and show a better modellization for bitter note both in model validation and prediction with a smaller error in bitter sensory score prediction i.e. 0.87 vs 1.60 for aroma-taste and aroma respectively.

Model parameters in validation and prediction	External Test Set		Bitter evaluation on External Test Set	
	Bitter	samples	meas	pred
SEP ^b	0.87	37	0.33	-0.04
PRESS	6.08	40	2.90	3.34
r pred ^a	0.92	53	0.83	2.13
Factors	1.00	75	0.18	0.56
Slope	0.96	79	3.30	3.12
Intercept	0.35	114	0.00	-0.84
r val ^c	0.95	BUK	6.47	6.29
SEV ^d	0.59	INDOAP	1.65	3.43

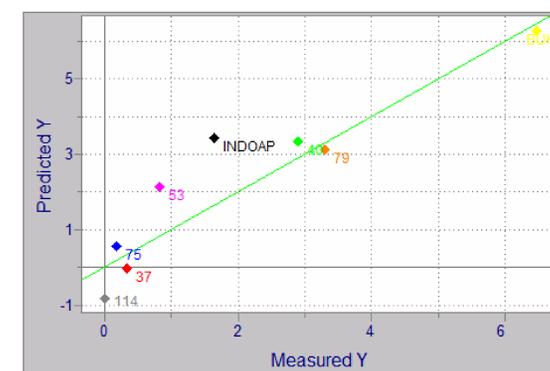


Figure 3 OPLS regression model for bitter scores prediction as an association measure between volatiles and taste fingerprinting and the sensory scores of 8 coffee samples. ^aCorrelation coefficient in prediction, ^bStandard error in Prediction, ^cCorrelation coefficient in Validation, ^dStandard error in Validation. Pareto scaling of raw normalized data.

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CONCLUSIONS

A flavoromic approach is here proposed to predict the bitter note of coffee. This approach revealed that several non-volatile and volatile compounds other than caffeine elicit the perception of bitter as representative of the multimodal effect. The non-volatile fraction studied most probably well represents for bitter note and could be of interest to see whether it is also related to the expression of other coffee aroma sensory attributes. The evaluation of the volatile fraction alone has shown phenol derivatives and pyrazines highly correlated with the bitter note with a good capability of the sensory score prediction, also if minor compared to the non-volatiles and fused data. Unlike the non-volatiles, although the chemical information on samples distribution are similar (MFA results), the study of the volatiles to correlate chemicals to the sensory attribute affords to investigate simultaneously different aroma attributes. In addition, the dedicated analytical instrumentation can easily be automatable and integrated (sample preparation, analysis and data mining) in an all-in-one system that could be useful at-line not only in a quality control laboratory but also to provide information complementary to those of the a sensory panel. The weak point in using aroma in prediction could be the error in prediction that may generate high false positive in samples acceptance; this limit can be overcome by a fine-tuning of the prediction model by analyzing a high number of samples together with a reliable alignment of the expert panel.