Dynamics of flavor perception: combining sensory methods and direct injection mass spectrometry

Sine Yener[1,2], Mathilde Charles[1], Andrea Romano[1,*], Luca Cappellin[1], Elisabetta Benozzi[1,2], Sofia Miori[1], Tillman D. Maerk[2], Luciano Navarini[3], Franco Biasoli[1], Flavia Gasperi[1]

[1]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), via E. Mach 1, 38010 San Michele all'Adige (TN), Italy
[2]Institut für Ionenphysik und Angewandte Physik, Leopold-Franzens Universität Innsbruck, Technikerstr. 25, 6020 Innsbruck, Austria
[3]illycaffè S.p.a, Via Flavia, 110, 34147 Trieste, Italy

Contact: *andrea.romano@fmach.it
mathildeclemence.charles@fmach.it

Introduction

-Volatile compounds play an important role in the perceived aroma of food. Direct injection mass spectrometry allows *in vivo* monitoring of volatile release in the nose. *Nosespace analysis (NA)* coupled with with Proton Transfer Reaction-Time of Flight- Mass Spectrometry (*PTR-ToF-MS*) is a promising new technique used in the last decade to study the relationship between flavor release and food properties [1].

-*Temporal Dominance of Sensations (TDS)* permits to describe the temporal evolution of the dominant sensations during product consumption. A dominant sensation is described as "a sensation catching the attention at a given time" [2].

In this study, a combination of TDS and NA with PTR-*ToF*-MS techniques were used to study the effect of roasting degree and sugar addition to espresso coffee on flavor perception and volatile release.

Material & Methods

Samples

- 2 types of coffee: A (light) and B (dark)
- 2 levels of sugar : 0 and 1g/10ml

10 ml served 55°C sipped with a straw

TDS Panel

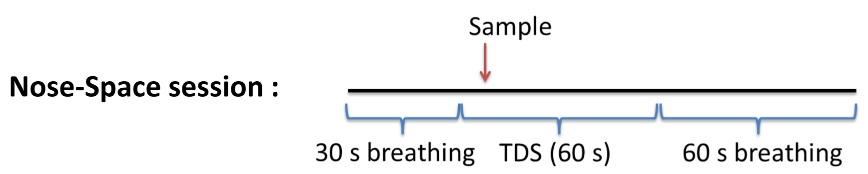
- 18 judges trained on TDS (F = 56% / M = 44% ; 23-37 year **old**)

TDS Method

- 9 attributes (***Taste and sensation:*** sweet, sour, bitter, astringent ; ***Aroma:*** roasted, burnt, caramel, nutty, vegetal)
- Evaluation
  - 60 seconds (putting the sample in mouth, swallowing after 5 s)
  - 3 samples/session – 10 min break between samples x 4 sessions
  - Presentation according a William's latin square (constraint to have 2 sweetened coffees + 1 unsweetened/session or the contrary)

Fig 1. Combination of TDS and Nose-Space analyses

NS analysis : Performed simultaneously to TDS (Fig 1).



Dead time correction, internal calibration of raw NS data and peak extraction were performed as described in elsewhere [3, 4].

- The NS data were scaled, peak-like features related to coffee were selected [1].
- The individual NS sessions were averaged and parameters related to single release curves were extracted: the maximum (*maximum*), the area under the curve (*area*), the median (median), the time to reach the maximum (*tmax*), the average of the last five seconds of the nosespace session (*final*), and the slope of the first descending section of the curve (*slope*), assuming a linear relationship between time and the logarithm of peak intensity [5].
- The columns containing NaN (Not a Number) cells are further eliminated from the matrix (Fig 2).
- The annotation of mass lists allowed assigning sum formulas and tentative identification of mass peaks.
- The data further analyzed with R (R foundation for statistical computing, Vienna, Austria).

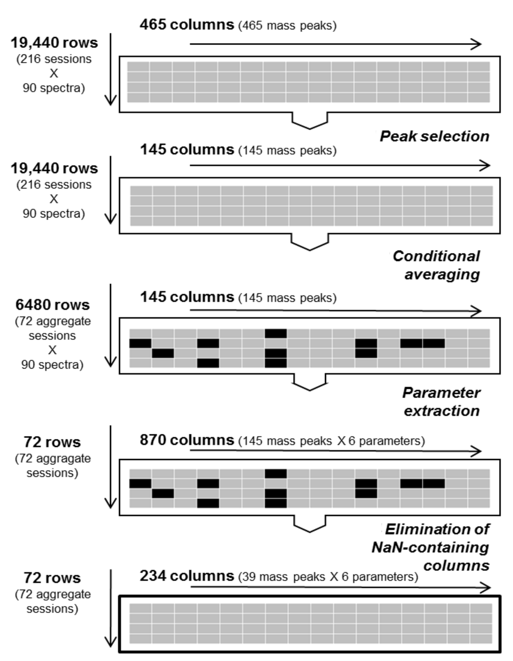


Fig 2. Protocol employed for NS data analysis (▦ = numeric cell; ■ = non numeric cell)

Results & Discussion

Dominance rates of TDS data (proportion of panelists who chose one attribute as dominant at a specific time) were calculated for each attribute and each product. The TDS curves obtained were represented on one graph per product (Fig 3). Two lines were drawn in the graphs to help comprehension of TDS data: 1) the "chance level" which corresponds to the dominance rate that an attribute can obtain by chance. Its value , P_0, is equal to 1/p, p being the number of attributes. 2) the "significance level" represents the minimum value that must be reached to the consider the dominant rate as significantly higher than P_0. This value is calculated following the confidence interval of a binomial proportion based on a normal approximation [6].

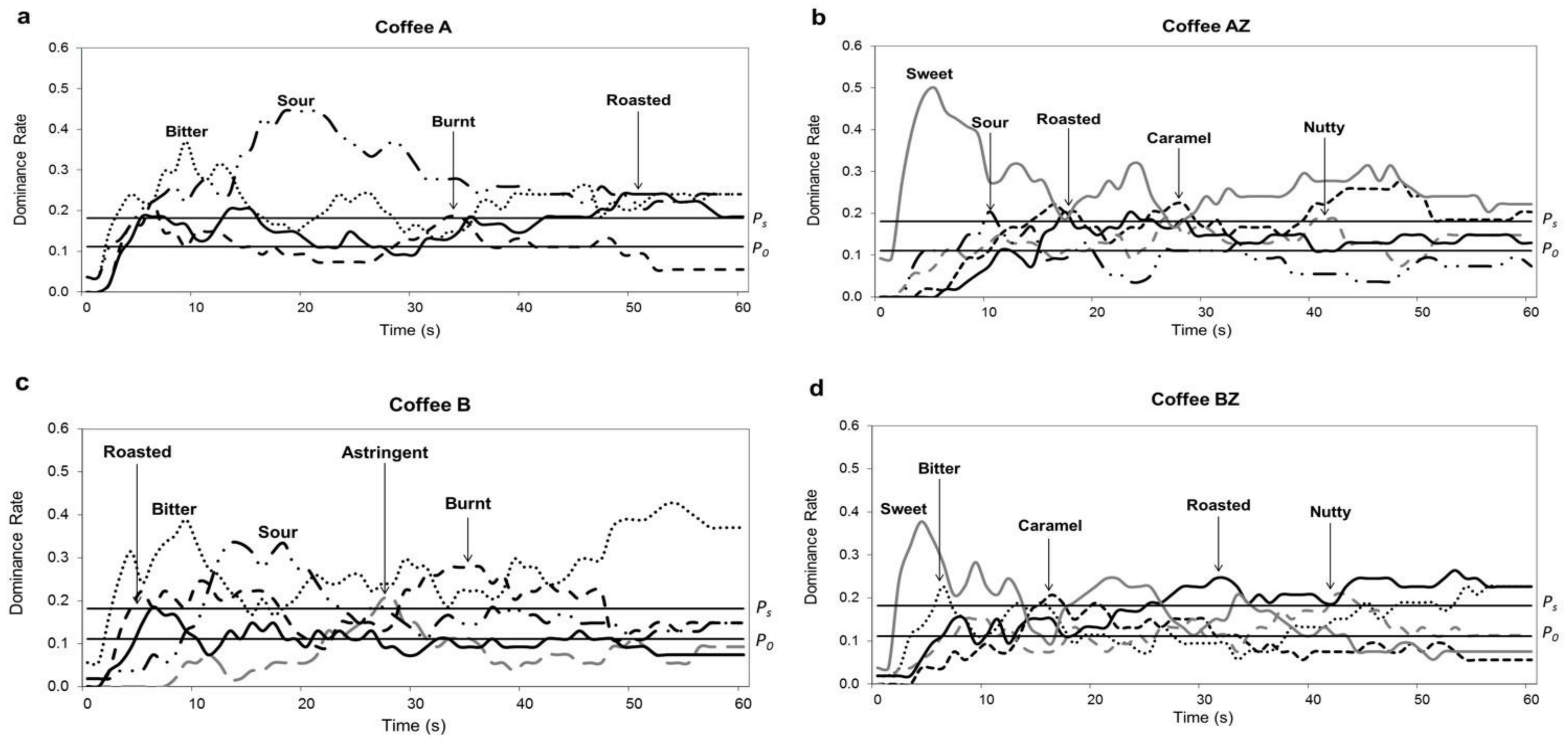


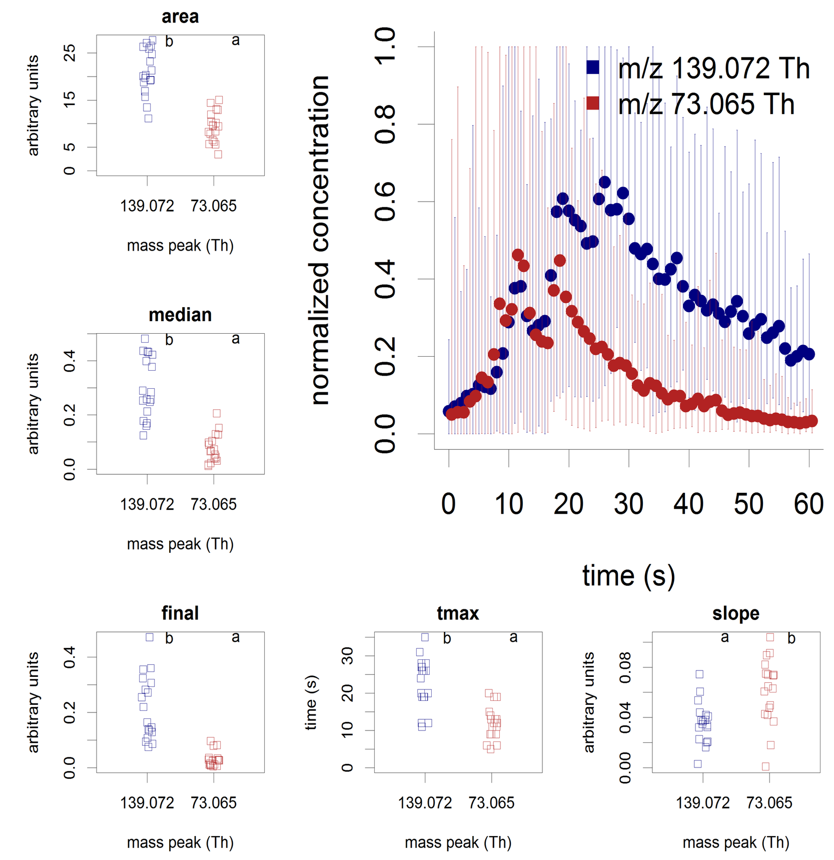
Fig 3. TDS curves of the different significant attributes for the four coffee samples over a 1 minute time period: Coffee A (a), Coffee AZ (b), Coffee B (c), Coffee BZ (d). P_0 represents the chance level and P_s is the significance level.

The TDS method allows differentiation of samples. Sugar addition (Fig 3) modifies the dominant attributes. In both coffees A and B, the fact to add sugar tends to mask/decrease sour and bitter taste dominance and to enhance the "positive aroma" perception described with the attributes *Caramel*, *Nutty* and *Roasted* instead of *Burnt.*

- Detailed analysis of NS PTR-ToF-MS data showed clustering of release curves into two distinct groups characterized by different patterns/time evolution in terms of physico-chemical basis (cluster 1 and 2) (on the right).
- The change in the dominant attribute in the different phases of coffee drinking: could be explained by an early and/or late onset of some mass peaks responsible for a sensory note: e.g. Possible markers of *Burnt* note (methyl-pyrrole (cluster 1) or acetyl- methyl-pyrrole (cluster 2)).
- Pyrazines (cluster 2) could be good temporal dominance markers of *Roasted* notes those could explain the increase between coffee A and B.
- The effect of sugar was more complex and difficult to explain the results because of different palette of sensory attributes used by the panel.

meas. mass (Th)	Coffees An	AZ	Bn	BZ	sum formula	chem. class	tentative identification
43.018	red	red	red	red	$C_2H_3O^+$	fragment	fragment (diverse origin)
45.034	red	red	red	red	$C_2H_5O^+$	aldehydes	acetaldehyde
49.012	red	red	red	red	CH_5S^+	sulfurs	methanethiol
61.029	red	red	red	red	$C_2H_5O_2^+$	acids/esters	acetic acid/methyl-formate
63.043	red	red	red	red	$C_2H_7O_2^+$	n.a.	non identified
68.050	blue	blue	blue	blue	$C_4H_6N^+$	N-heterocycles	Pyrrole
69.033	red	red	red	red	$C_4H_5O^+$	furans	Furan
71.049				blue	$C_4H_7O^+$	fragments/aldehydes/ketones	fragment (methyl-butanol)/butenal/butenone
73.065	red	red	red	red	$C_4H_9O^+$	aldehydes/ketones	isobutanal/butanone
75.044	red	red	red	red	$C_3H_7O_2^+$	esters/hydroxyketones	methyl-acetate/acetol
78.968	red	red	red	red	n.a.	n.a.	non identified
80.049			blue	blue	$C_5H_6N^+$	N-heterocycles	pyridine
81.034	blue	blue	blue	blue	$C_5H_5O^+$	fragments	furan fragment
82.065	red	red	red	red	$C_5H_8N^+$	N-heterocycles	methyl-pyrrole
83.049	red	red	red	red	$C_5H_7O^+$	furans	methyl-furan
85.064	red	red	red	red	$C_5H_9O^+$	aldehydes	methyl-butenal
87.043	red	red		red	$C_4H_7O_2^+$	ketones	butanedione/butyrolactone
87.080	red	red	red	red	$C_5H_{11}O^+$	aldehydes	methyl-butanal
89.059			red	red	$C_4H_9O_2^+$	esters/hydroxyketones	methyl-propanoate/hydroxy-butanone
94.039	blue	blue	blue	blue	n.a.	n.a.	non identified
95.010	red	red	red	red	n.a.	n.a.	non identified
97.027	blue		blue	blue	$C_5H_5O_2^+$	furans	furfural
98.060	blue		blue	blue	$C_5H_8ON^+$	N-heterocycles	dimethyl-oxazole
99.041	blue	blue	blue	blue	$C_5H_7O_2^+$	furans/lactones	furfuryl alcohol/angelica lactone
99.079	red		red		$C_6H_{11}O^+$	aldehydes/ketones	hexenal/methyl-pentenone
100.020	blue	blue	blue	blue	$C_4H_6NS^+$	N-heterocycles	methyl-thiazole
101.058	red	red		red	$C_5H_9O_2^+$	Ketones	pentanedione/methyl-tetrahydrofuranone
103.072	blue	blue	blue	blue	$C_5H_{11}O_2^+$	esters/hydroxyketones	hydroxy-pentanone/methyl-butanoic acid
105.068	red	red	red	red	$C_8H_9^+$	aromatic hydrocarbons/fragments	styrene/phenylethanol fragment
109.071	blue	blue	blue	blue	$C_6H_9N_2^+$	N-heterocycles	dimethylpyrazine/ethylpyrazine
111.042	blue		blue	blue	$C_6H_7O_2^+$	furans	acetyl_furan/methyl-furfural
113.056	blue	blue	blue	blue	$C_6H_9O_2^+$	mixed	methyl-furfuryl-alcohol/dimethyl-furanone methyl-cyclopentanedione/cyclotene
115.072	red	red	red	red	$C_6H_{11}O_2^+$	pyrans	4-methyltetrahydro-2H-pyran-2-one
124.072	blue	blue	blue	blue	$C_7H_{10}ON^+$	N-heterocycles	2-acetyl-1-methylpyrrole
125.057	blue	blue	blue	blue	$C_7H_9O_2^+$	phenols/furans	guaiacol/methyl-benzenediol/furyl acetone
139.072	blue	blue	blue	blue	$C_8H_{11}O_2^+$	phenols	4-ethyl-1/2-benzenediol
141.056				blue	$C_7H_9O_3^+$	furans	furfuryl-acetate
148.069	blue	blue	blue	blue	n.a.	n.a.	non identified
149.058			red	red	$C_9H_9O_2^+$	furans	furfuryl-furan

n.a. = non applicable



- The difference in release patterns was also reflected by the extracted parameters from the individual NS curves, with normalized curves from cluster 2 having higher *area, median, final* and *tmax*, and lower *slope*, than those from cluster 1 (Fig 4).
- The impact of sugar addition on volatile release appeared to be negligible (one-way ANOVA, *p<0.01*).

Fig 4. Comparison between peaks from cluster 1 (blue) and cluster 2 (red), measured in coffee BZ. The normalized release curves show mean, maximum, and minimum values (dots and error bars). Stripcharts display the distribution of single values for the curve parameters, with letter annotations indicating statistically significant differences (one-way ANOVA, *p*<0.01). *m/z* 73.065 (attributed to isobutanal/butanone) and mass peak *m/z* 139.072 (tentatively assigned to 4-ethyl-1,2-benzenediol).

Conclusions

- *The simultaneous application of TDS and NS analysis allowed differentiation of products with a marked effect of roasting.*
- *NS analysis can identify differences between panelists and provide a better understanding of sensory data.*
- *Addition of sugar modified sensory profiles of coffees in terms of selected attributes but no significant effect was observed on aroma release underlying the presence of cognitive multisensory interactions.*
- *The grouping of volatile compounds according to their release characteristics can be related to explain the changes in aroma perception in TDS analysis.*

References

[1] Romano et al., 2013. International Journal of Mass Spectrometry 365-366:20-27.
[2] Pineau et al. 2012. *Food Quality and Preference* 26 :159-165.
[3] Cappellin et al., 2010. International Journal of Mass Spectrometry 290:60-63.
[4] Cappellin et al., 2011. Sensors and Actuators B: Chemical 155:183-190.
[5] Normand. V., 2010. Chem. Senses 29:235-245.
[6] Pineau et al., 2009. *Food Quality and Preference* 20:450-455.

Acknowledgements

Sine Yener is a fellowship holder from **PIMMS (Proton Ionisation Molecular Mass Spectrometry) ITN** supported by the European Commission's 7th Framework Programme under Grant Agreement Number 287382.