



AperTO - Archivio Istituzionale Open Access dell'Università di Torino

A Further Tool To Monitor Coffee Roasting Process: Aroma Composition And Chemical Indices

This is the author's manuscript
Original Citation:
Availability:
This version is available http://hdl.handle.net/2318/123524 since 2016-07-14T11:43:47Z
Published version:
DOI:10.1021/fj3031716
Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on: [Erica Liberto, Manuela R. Ruosi, Chiara Cordero , Patrizia Rubiolo, Carlo Bicchi, Barbara Sgorbini,

Non-separative headspace solid phase microextraction-mass spectrometry profile as a marker to monitor coffee roasting degree, JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, Volume: 61 Issue: 8 Pages: 1652-1660 Published: FEB 27 2013, 10.1021/jf303067q, ACS]

> *The definitive version is available at:* [http://pubs.acs.org/doi/full/10.1021/jf303067q]

- 1 Non-separative headspace solid phase microextraction-mass spectrometry profile as a marker
- 2 to monitor coffee roasting degree
- 3 Erica Liberto^{1*}, Manuela R. Ruosi¹, Chiara Cordero¹, Patrizia Rubiolo¹, Carlo Bicchi¹, Barbara Sgorbini¹,
- ⁴ ¹Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, I-10125 Torino, Italy
- 5 *Address for correspondence:
- 6 Dr. Erica Liberto Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via P. Giuria 9, I-
- 7 10125 Torino, Italy e-mail: erica.liberto@unito.it; phone: +39 011 670 7662; fax: +39 011 670 7687

9 Abstract

10 This study describes a non-separative Headspace-Solid-Phase-Microextraction-Mass-Spectrometry (HS-SPME-MS) approach, in view of its application to on-line 11 monitoring of a roasting process. The system can quickly provide representative and 12 diagnostic fingerprints of the volatile fraction of samples and, in combination with 13 appropriate chemometric pattern-recognition and regression techniques, 14 can successfully be applied to characterize, discriminate and/or correlate patterns with the 15 roasting process. Eighty coffee samples of different varieties, geographical origins 16 and blends were analyzed. The experimental HS-SPME-MS results show that the TIC 17 fingerprint can be used to discriminate the degree of roasting; diagnostic ion 18 abundance(s) or ratios were closely correlated with the roasting process; both could 19 successfully be used as markers or Analytical Decision Makers, to monitor roasting 20 processes on-line, and to define quality and safety of roasted coffee. 21

22

KEYWORDS: HS-SPME-MS; MS-EN; coffee; aroma quality and safety;
 technological process; roasting indices; on-line monitoring

26 INTRODUCTION

27 The roasting process is a significant factor in determining coffee flavor. In particular, coffee aroma depends on the specific quali-quantitative distribution of various 28 components. These are mainly volatile medium-to-high polarity compounds, deriving 29 from drying and from the heat-browning related to the roasting conditions, above all 30 temperature and time. The aroma also depends on the species, variety and blend, as 31 well as on geographical origin¹⁻⁴. Conversely, the roasting process can also produce 32 some compounds that must be monitored because of their toxic properties; this is the 33 case of furan and its derivatives, which have shown carcinogenic and cytotoxic 34 activities⁵⁻⁸. The reduction of furan in coffee is therefore highly recommended, and 35 may be achieved by optimizing the roasting process in all its steps (i.e. roasting, 36 cooling, degassing and grinding) while, obviously, keeping the organoleptic 37 properties unaltered. 38

The effect of the roasting process on coffee beans is generally described in terms of 39 the degree of roasting, and is usually evaluated through several chemical and physical 40 parameters, including external color of the beans, loss of weight during roasting, and 41 variations in chemical composition, as well as through the development of sensory 42 characteristics⁹⁻¹⁰. These parameters concur to define the degree of roasting, although 43 44 to date a concise, clear and universally-accepted evaluation protocol does not exist. The most widely-used parameter to determine degree of roasting in day-to-day 45 practice is color, measured through the light reflectance of ground beans or, still 46 47 today, by visual inspection. This latter method is still valid partly because the industry uses a constant quality and variety of green coffee, combined with constant 48 time-temperature conditions and roasting plant. Dry-matter loss is also considered to 49 provide a reliable and complementary evaluation of the degree of roasting, including 50 for in-plant determination⁹. Correct conditions for industrial roasting processes are, in 51 general, obtained by appropriately scaling-up the results of experiments from pilot or 52 laboratory plants monitored by physical methods. 53

This study describes a non-separative headspace solid phase microextraction-mass 54 spectrometric method (HS-SPME-MS), in view of its possible application to the on-55 line monitoring of roasting processes. HS-SPME is a high-concentration-capacity 56 technique offering good recoveries and easy to automate, that can be combined 57 directly on-line with mass spectrometry¹¹. Non-separative MS methods, better known 58 as mass spectrometry-based electronic nose (MS-EN)¹²⁻¹⁶, were introduced by 59 Marsili¹⁷ to study off-flavors in milk; they have since been applied successfully to 60 characterizing several matrices, in particular in the food field¹⁸⁻²⁰. They provide a 61 representative, diagnostic and generalized mass spectrometric fingerprint of the 62 volatile fraction of a sample, analyzed directly without prior chromatographic 63 separation, in which each m/z ratio acts as a "sensor" whose intensity derives from 64 the contribution of each compound producing that fragment. These methods, in 65 combination with appropriate chemometric elaboration can be used to quickly 66 characterize and discriminate samples within a set, and to correlate them with a 67 technological process (e.g. coffee roasting). MS-EN can also be used to monitor 68

target compounds in a group of samples, provided that specific and diagnostic ionsare obtained with a compatible ion generation mode (EI, CI, APCI, PTR etc.).

71 Mass spectral fingerprints, or diagnostic ion abundance(s), were here used both as

marker and as analytical decision maker $(ADM)^{21}$ to monitor coffee roasting degree,

73 in view of the possibility of combining a mass spectrometer directly on-line to a

74 roasting machine. In particular, the results are reported of a study aimed at correlating

75 HS-SPME-MS profile and coffee color, as a further tool to characterize roasting

76 processes.77

78 MATERIALS AND METHODS

79 Samples:

Eighty roasted-coffee samples from different geographical origins, of different 80 varieties and blends (100% Arabica, 100% Robusta, 50/50% Arabica-Robusta blend, 81 and several commercial blends) at different degrees of roasting were supplied by 82 Lavazza (Turin - Italy) over a period of 12 months. Colors of samples were 83 measured, by ground-bean light reflectance, with a single-beam Neuhaus Neotec 84 Color Test II instrument, at wavelength 900 nm, on 25-30g of ground coffee. Table 1 85 gives the list of samples with their color values. A set of 100 coffee pods from the 86 same lot (a 50/50 Arabica and Robusta blend) for espresso machines was stored at -87 18°C and used as reference to standardize the HS-SPME system performance over 88 89 time.

Headspace Solid Phase Microextraction (HS-SPME) sampling and GC analysis conditions

93 The SPME device and fibers were from Supelco (Bellefonte, PA, USA).

- 94 Divinylbenzene/Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS) $d_f 50/30 \mu m$, 2 95 cm long fibers were used, conditioning them before use as recommended by the 96 manufacturer²².
- 97 Volatiles were sampled by automated headspace Solid Phase Microextraction (auto-98 HS-SPME) using a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, 99 Germany) on-line integrated with an Agilent 7890 GC unit coupled to an Agilent 100 5975 MS detector (Agilent, Little Falls, DE, USA). 500 mg of ground roasted coffee 101 in a 20mL screw-cap vial were sampled by HS-SPME at 50°C for 10 minutes¹⁰. All 102 samples were analyzed in triplicate. Three fibers were selected from different lots, 103 after preliminary testing to establish their sampling performance, so as to select
- 104 equivalent fibers for use throughout the entire analysis period (see below: HS-SPME 105 fiber performance evaluation). Fiber performance was monitored throughout the
- 106 study on an additional set of coffee pod samples, and on 5uL of a 2 mg/mL standard 107 solution of α - and β -thujone in dibutyl phthalate.
- 107 solution of α and β -thujone in dibutyl phthalate. 108 *Non-separative analysis* – *GC unit conditions:* oven and injector temperature: 250 °C 109 injection mode: split, split ratio: 1/10; carrier gas: helium, flow rate: 0.4 mL/min;
- fiber desorption time and reconditioning: 3min; transfer column: deactivated fused silica tubing (d_c 0.10mm, length 6.70m) (Mega, Legnano (Milan), Italy).
- *MSD conditions*: ionization: EI mode at 70 eV; transfer line: 280°C. Standard tuning was used and the scan range was set at m/z 35-350 with a scanning rate of 1.000 amu/s.
- 115 Separative GC-MS analysis Chromatographic conditions: injector temperature:
- 116 230°C, injection mode: split, ratio: 1/10; carrier gas: helium, flow rate: 1 mL/min;
- fiber desorption time and reconditioning: 5 min; column: MEGAWAX 20M (d_f 0.20 μ m, d_c 0.20 mm, length 50 m) (Mega, Legnano (Milan), Italy). Temperature program:
- 119 from 40°C (1 min) to 200°C at 3°C/min, then to 250 °C (5min) at 10°C/min.
- *MSD conditions:* ionization mode: EI (70 eV), scan range: 35 to 350 amu; ion source
 temperature: 230°C; quadrupole temperature: 150°C; transfer line temperature:
 250°C.
- 123 Data were acquired and processed with an Agilent MSD Chem Station ver 124 E.02.01.1177. (Agilent, Little Falls, DE, USA)
- 125 Analytes were identified by their linear retention indices and EI-MS spectra, by
- 126 comparison with authentic standards, or were tentatively identified through their EI-
- 127 MS fragmentation patterns and retention indices.
- 128

129 Chemometric analyses

- 130 Principal Component Analysis (PCA) and Orthogonal Partial Least Square analysis
- 131 (OPLS) were performed with Pirouette software ver. 4.0 (Infometrix, Inc. Bothell,
- 132 WA). The software package was used to automatically create ASCII files from
- Agilent GC ChemStation data, using a post-run macro. The data matrix consisted of
- 134 as many rows as the number of samples (total objects: 240) and 316 columns (m/z

variables). Samples were randomly divided into a training set (55 samples) and a test set (25 samples). PCA was used in the first step for pattern recognition analysis, to visualize information and sample clusters, in particular as a function of the technological process. OPLS analysis was then carried out to correlate color (as a marker of the degree of roasting) to the chemical fingerprint. Data were pre-treated by baseline correction, through noise subtraction, and by internal normalization of the signal from each sample; they were subsequently pre-processed by mean-centering.

141 signal from each sample; they were subsequently pre-processed by 142

143 **RESULTS AND DISCUSSION**

144 Compared to separative GC-MS profiles, MS-EN patterns provide a fast response; however, the significance of the TIC is low, and less information can apparently be 145 obtained from the MS profile. This is because the intensity of each fragment (m/z) is 146 "built up" from the contributions of each component of the sample involved in 147 generating that ion during its ionization process. Figure 1 gives the HS-SPME-GC-148 MS (a) and the HS-SPME-MS TIC (b) profiles, together with the mass spectral 149 fingerprint (c) of an Arabica coffee sample. Table 2 lists the components identified in 150 the HS-SPME-GC-MS profile of the same Arabica sample, together with their target 151 (TI) and qualifier ions (Qi). 152

- When applying HS-SPME-MS, it is mandatory to use chemometric techniques to 153 "extract" data from the MS profile that can provide significant information. However, 154 when a mathematical model is generated and used to classify or correlate 155 composition or characteristics of a sample, and to reveal the resources of hidden 156 information, one of the main limits is profile precision over a long period. This 157 variability can reduce the effectiveness of a successful model, generated with a 158 training set, when applied to routine analyses. The most critical points in the case of 159 HS-SPME-MS are SPME fiber performance and MS signal instability; the latter may 160 be due to ion source contamination, aging of electron multiplier, and/or filament 161 electron emission. These limits can be overcome by i) evaluating the response of a 162 suitable number of SPME fibers and monitoring it continually, ii) checking electron 163 multiplier response over time, and iii) standardizing the MS profile through internal 164 normalization. 165
- 166

167 HS-SPME fiber performance evaluation

SPME fiber effectiveness was evaluated initially in terms of total fingerprint areas: 168 this was aimed at minimizing sampling errors/discriminations, in view of the 169 extended time interval (12 months) covered by this study, and of the large number of 170 samples and replicates expected to be run. As discussed elsewhere²¹, the sampling 171 performance of three DVB/CAR/PDMS fibers from different lots was tested on 172 coffee pod samples and on an α - and β -thujone standard solution, to classify them on 173 the basis of the recovery provided, and to monitor any change over time. Normalized 174 spectral fingerprint areas of the coffee volatile fraction of five replicates from the 175 same pod for each fiber (F1, F2, F3) were submitted to Analysis of Variance 176 (ANOVA). The One-Way ANOVA results on the replicates for each fiber are in table 177 S1 (supporting information), and showed that the null hypothesis ("there is no 178

179 difference among the fibers in terms of absolute peak areas of the selected target 180 analytes") was false. Tukey's test classified F1 and F3 as belonging to the same 181 group, and these were therefore adopted for all experiments. Similar results were 182 obtained with α - and β -thujone standard solution. When additional fibers were 183 necessary, they were submitted to the entire test routine, analyzing reference coffee-184 pod samples. Normalized spectral fingerprint area values had to fall within 5% 185 variability (expressed as RSD%) as established during performance testing.

186

187 **Precision and internal normalization**

Precision. expressed as repeatability and intermediate precision of HS-SPME-MS on 188 reference coffee-pod samples, was evaluated over the entire period. Repeatability was 189 calculated over five analyses of coffee in the same pod, the MS profile fragments of 190 each sample were normalized *versus* the most intense ions (m/z 43, basic peak) taken 191 as 1; each m/z intensity value is expressed as a percentage of the intensity of the basic 192 m/z fragment^{14,24}. Repeatability is expressed as Relative Standard Deviation % 193 (RSD%) on the total areas of the normalized fingerprints, and on some diagnostic m/z 194 ions characteristic of certain components related to roasting, aroma or toxic 195 chemicals. Intermediate precision was calculated as before was calculated as 196 197 described above?// was calculated as described elsewhere? over five analyses of the coffee pod, carried out monthly over a period of one year. Results are in table S2 198 (supporting information) and show very good intermediate precision, with RSD% 199 values for the total fingerprinting area of 3.16%, and for single ions ranging from 200 4.65% for m/z 45 to 20.08% for m/z 150. 201

202

203 Unsupervised exploratory analysis

Although color is the most widely-adopted parameter used to monitor the roasting 204 process, on-line monitoring of the development of the volatile fraction by means of 205 an analytical approach (HS-SPME-MS) could be very useful to improve coffee 206 standardization and to optimize its aroma. Fifty five coffee samples of different 207 varieties and blends, and roasted to different degrees, i.e. dark (color range: 35÷42), 208 medium (46÷53) and light (57÷62), were analyzed. Their spectral fingerprints were 209 processed using an unsupervised approach, Principal Component Analysis (PCA), 210 with the aim of finding inter-sample and inter-variable relationships with color (e.g. 211 degree of roasting). This was done by visualizing spots? variables? scores? 212 components?—sample distribution on the score plots resulting from exploratory 213 analysis³¹⁻³³. PCA clearly showed that coffee samples are discriminated by their 214 roasting degree (color) on the first principal component (1st PC), while different 215 varieties are separated on the 2^{nd} and 3^{rd} PCs (Figure 2a). The volatile fraction 216 included the well-known Maillard reaction products, derived from non-volatile 217 precursor degradation (Amadori compounds and deoxyosones) namely furanes, 218 furanones and pyranones. Compounds resulting from the Strecker reaction of α -219 dicarbonyls and aminoacids were also present: 2,3-butandione, 2- and 3-220 methylbutanal and alkyl-pyrazines, as were sugar degradation products (furfural, 5-221 methylfurfural and furfuryl alcohol). The loading plot (figure 2b) indicates those m/z 222

fragments that vary linearly with roasting degree; this indirectly also shows which 223 components change during roasting: m/z 52 and 79 (mainly representative of pyridine 224 and furfuryl acetate), 81 (1-methyl-pyrrole, 1-furfuryl-pyrrole and furfuryl acetate), 225 98 (furfuryl alcohol), 108 (dimethyl pyrazine groups: 2,3-, 2,5-, 2,6-isomers), 109 226 (guaiacol), 110 (5-methyl furfural), and 96 (furfural) all have high relevance// are all 227 highly significant? on the 1st PC (explained variance 59.90%), and can be taken as 228 markers of roasting, as they increase markedly with the roasting degree. Some of 229 them are also key-aroma components, and are marked with an asterisk in Table 230 S2^{25,26}. M/z 43 (2,3-hexandione, acetoxyacetone), 45 (acetoine and 3-hydroxy-2-231 butanone), 57 (2-oxopropyl propanoate), 60 (acetic acid), 79 (pyridine), and 95-96 232 (acetyl furan, furfural) on the 2nd PC (exp. var. 23.31%) are markers of variety and, 233 roasting degree being equal????, are more abundant in Arabica than in the blend or 234 Robusta samples; they decrease with roasting time/temperature. Conversely, m/z 108 235 (mainly deriving from dimethyl pyrazines) and 109 (guaiacol) are more intense in 236 Robusta samples. These considerations confirm the findings of a study by Ruosi et al. 237 using separative techniques.²⁷ 238

Some m/z variables vary linearly with roasting degree, for example furan and its 239 homologues. Their formation is related linearly to the degree of roasting, as shown in 240 figure 3; determination coefficients were 0.7754 and 0.8917 for furan and 2-241 methylfuran, respectively. Despite the presence of interference by the same m/z 242 fragment from other origins, this close correlation with degree of roasting means that 243 furan and 2-methyl furan formation can be monitored during roasting via their 244 characterizing ions (target ions), i.e. 68 and 82. If the intensities of these ions are 245 outside fixed limits of acceptance, the relative compounds can be quantified by 246 conventional methods ^{28,29}. In addition to conventional separative methods, in 2011 247 Bicchi et al. proposed a quick method to quantify these compounds by HS-SPME-248 MS; results were comparable to those of conventional methods 30 . 249

250

251 "Supervised" multivariate regression

PCA results show that the MS profile of the coffee volatile fraction is closely 252 correlated to the degree of roasting, thanks to some variables (m/z values, and the 253 originating *#* the relative? components) that are characteristic of this technological 254 process. Orthogonal Partial Least Square analysis (OPLS) was thus used as a measure 255 of correlation, i.e. to evaluate the closeness of association between the fingerprint of 256 the volatile fraction of coffee and its color, as an indicator of technological treatment, 257 rather than for its ability to predict coffee color ^{33,34}. The OPLS model requires a 258 training set to demonstrate any correlation between coffee color and aroma. Training 259 (55) and test (25) sets of samples, selected randomly and consisting of several 260 commercially-available Arabica (100%) and Robusta (100%) coffees of different 261 origins, plus blends of the two at different percentages, were established. 262

The OPLS regression model was first calculated on 5 PCs, and internally crossvalidated on the training set data relating to the volatile fraction spectral fingerprint and coffee color; the model was then applied to the test set samples. The results showed a highly negative linear correlation between measured (i.e. all m/z fragments 267 of the volatile fraction of coffee samples) and predicted (color values) variables. The correlation between spectral fingerprint and color is negative due to how color values 268 are expressed, since high color values indicate lightly-roasted samples. //In terms of 269 predictive value, there was a close correlation (rpred 0.9472) with a satisfactory 270 Standard Error of Prediction (SEP 2.53); values were within the range of colors that 271 discriminate the different degree roasting (i.e. light, medium and dark). TEMO DI 272 NON AVER FATTO GIUSTO, CI RIPROVO// When used in prediction mode, the 273 correlation value was high (rpred 0.9472) with a satisfactory Standard Error of 274 Prediction (SEP 2.53); values were within the range of colors that discriminate the 275 different degree roasting (i.e. light, medium and dark). 276 Figure 4 shows the association intensity relationship between measured and predicted 277

color values, using the model built with the training set, and gives the parameters of 278 the validated model and of the prediction. As shown by PCA, the m/z ions with good 279 correlation values (> \pm 0.7) are related to compounds whose abundance is affected 280 markedly by the roasting process, and that are linked to the aroma developing during 281 this process (Fig 2b). The volatile fraction spectral fingerprint/color correlation is 282 important to monitor the roasting process because, besides establishing a relationship 283 between chemical and physical data, it (sarebbe the correlation: OK?) can give 284 specific chemical information about aroma changes with technological treatments. 285

286

Identification of indices of the degree of roasting and possible relation with the utility of roasted coffee color

This part of the study investigated the possibility of identifying reliable chemical 289 indices to be used for roasting process control, as a function of some informative m/z 290 ion ratios. If correctly monitored on the laboratory scale, through objective and 291 measurable indices, the roasting process could be directed so as to obtain the required 292 aroma profile, in particular when new blends are being developed. Starting from the 293 above PCA results, the two-by-two normalized area ratios of ions closely correlated 294 to the roasting process were calculated, using a specific visual basic Excel macro. 295 The resulting indices were multiplied by 1000, to facilitate their handling, and were 296 arbitrarily considered significant only when there was a difference of at least 100 297 units between light, medium and dark roasting degree, in each variety or blend. Three 298 indices (i.e. m/z ratios) were found to be in common among Arabica, Robusta and 299 blends: 79/110; 97/110; 98/110; these fragments are characteristic, among others, of 300 pyridine, 5-methyl furfural, 1-methyl pyrrole, furfuryl pyrrole and furfuryl alcohol. 301 Each fragment varied linearly with color and, as a consequence, with roasting degree. 302 Table 3 gives correlation equations and coefficients, between indices and color, for 303 each variety/blend, together with the average index values and related standard 304 deviation. These index values can successfully be used to discriminate the degree of 305 roasting with good confidence, as is shown by their standard deviations that, within 306 the same variety, avoid any risk of two index intervals deriving from two different 307 roasting treatments (e.g. medium or dark) overlapping. 308

These results are particularly interesting for industrial applications, where in general the material being processed comprises blends of green coffees with similar or 311 unvaried characteristics (variety, origins etc.); they enable a physical parameter 312 (color) to be correlated to chemical markers (indices) for the purpose of monitoring 313 the roasting process. Although the number of samples and varieties tested in the 314 present study is too small to be representative, these results outline the possibility of 315 defining indices of roasting and significant values for on-line process monitoring.

In addition, robust and reliable mathematical models to predict color directly from the 316 total spectral fingerprint and/or index values can successfully be applied on-line, not 317 only to predict color, but also to connect it to the aroma composition. A PLS model 318 equation was built and internally cross-validated with the 55 samples of the training 319 set and verified with the 25 samples of the test set (table 4). The results show a close 320 correlation between indices (i.e. m/z ratios) and color, with 17 of 25 samples having a 321 residual color measure below \pm 3, and only two of them (C21 and C24) presenting a 322 difference between measured and predicted color of approximately- 9. Table 4 323 reports the PLS results, together with the parameters of the model equation, and 324 shows a close correlation within the training set (rval: 0.9399) and a very satisfactory 325 standard error in color validation (SEV: 2.68). When the model equation was applied 326 to the samples of the test set, as expected the correlation coefficient in prediction 327 decreased (rpred: 0.8416) and the standard error in prediction increased (SEP: 4.29), 328 although the values were still satisfactory. These results show a reliable correlation, 329 although a less uniform training set would be necessary to include a wider range of 330 variables (variety, origin, roasting conditions) in a single equation. The results are 331 very satisfactory if the diversity represented is considered, because the samples of the 332 training set were very different and their number was relatively small. 333

In conclusion, the results show that HS-SPME-MS for on/in-line control of coffee 334 roasting process is a promising approach, through which not only the evolution of the 335 total MS profile can be studied, but also specific ions or ion ratios. The quick non-336 separative method (HS-SPME-MS) described shows that a correlation between 337 spectral fingerprinting or roasting indices and color can be found, and that chemical 338 parameters can be used reliably to evaluate the degree of roasting. The combination 339 of MS profile and chemical indices with physical parameters affords more reliable 340 off-line monitoring or optimization of the roasting process, in particular to control 341 aroma development when developing new blends, and to detect the formation of toxic 342 compounds. In addition, the reliability of these results may be exploited as a 343 reference to validate those obtained by coupling a laboratory roasting machine to a 344 mass spectrometer directly, for on-line monitoring of the roasting process and marker 345 development³⁵⁻³⁸ HS-SPME-MS can also be used as analytical decision maker, i.e. to 346 select those sample(s) that have to be analyzed by conventional separative methods, 347 for instance when the non-separative intensities of some m/z fragments, diagnostic of 348 certain components, are outside the fixed limits of acceptance, or when the aroma 349 profile is not in line with that of the desired final product. Further studies are now 350 under way to extend the applicability of this method from model experiments to real-351 world samples. 352

353

354 ACKNOWLEDGMENT

The results here reported are part of the "ITACA" project of the POR-FESR "Competitività regionale e occupazione" 2007/2013, Asse 1, Misura I.1.1, "Piattaforme innovative" of the Piedmont Region (Italy), to which the authors are indebted.

359

360 SUPPORTING INFORMATION AVAILABLE

One-way Anova results on fiber performance evaluation of the total volatile fraction,
 spectral fingerprint areas of coffee pod samples, and method precision over 12
 months, are included as supporting material. This material is available free of charge

- 364 via the internet at <u>http://pubs.acs.org</u>.
- 365 LITERATURE CITED
- ¹ Flament, I. *Coffee Flavor Chemistry*; Wiley: Chichester, U.K., 2002.
- ² Nebesny, E., Budryn, G., Kula, J., Majda, T. The effect of roasting method on headspace composition of
- 368 Robusta coffee bean aroma. *Eur. Food Res. Technol.* **2007**, 225, 9-19.
- 369 ³ Moon, J., Shibamoto, T. Role of roasting conditions in the profile of volatile flavour chemicals formed from
- 370 coffee beans. J. Agric. Food Chem 2009, 57, 5823-5831.
- 4 Cordero, C., Liberto, E., Bicchi, C., Rubiolo, P., Reichenbach, S.E., Tian, X., Tao, Q. Targeted and non-
- targeted approaches for complex natural sample profiling by GC×GC–qMS. *J Chromatogr Sci.* **2010**, 48, 1-12.
- ⁵ Furan in foods-thermal treatment, US Food and Drug Administration (FDA), Washington, DC, 2004.
- 374 <u>www.cfsan.fda.gov/<lrd/fr040510.html</u> (27/05/2012).
- ⁶ Report of the scientific panel on contaminants in the food chain on provisional findings on furan in food,
- 376 European Food Safety Authority (EFSA), EFSA J. 2004 137, 1. Brussels, Belgium;
- 377 <u>www.efsa.eu.int/science/contam/contam documents/760 en.html</u> (27/05/2012).
- 378 ⁷ Crews, C., Castle, L. A review of the occurence, formation and analysis of furan in heat-processes foods.
- 379 Trends Food Sci. Technol. 2007, 18, 365-372.
- ⁸ IARC, Monographs on the evaluation of carcinogenic risks to human and their supplements **1995**, 63, 393.
- 381 ⁹ Clarke, R. J., Macrae, R. In *Coffee: Vol 2 Technology.*; edition 1; R. J. Clarke & R. Macrae Eds., Essex:
- 382 Elsevier Applied Science, **1987**.
- 383 ¹⁰ Baggenstoss, J., Poisson, L., Perren, R., Escher, F. Coffee roasting and aroma formation: application of
- different time-temperature conditions. *J. Agric. Food Chem.* **2008**, 56, 5836-5846.

- ¹¹ Arthur, C. L., Pawliszyn, J. Solid phase microextraction with thermal desorption using fused silica optical
 fiber. *Anal. Chem.* **1990**, 62, 2145-2148.
- ¹² Fenaille, F., Visani, P., Fumeaux, R., Milo, C., Guy, P.A., Comparison of mass spectrometry-based
 electronic nose and solid phase microextraction gas chromatographys-mass spectrometry technique to assess
 infant formula oxidation. *J. Agric. Food Chem.* 2003, 51, 2790-2796.
- ¹³ Pilar Marti, M.; Busto, O.; Guasch, J. Application of headspace mass spectrometry system to the
 differentiation and classification of wines according to their origin, variety and ageing. *J. Chromatogr. A* 2004,
 1057, 211-217.
- ¹⁴ Pavón, J. L. P., Sanchez, M.; Del Nogal; Pinto, C. G.; Laespada, E. F.; Cordero, B. M.; Pen⁻ a, A. G.
 Strategies for qualitative and quantitative analyses with mass spectrometry-based electronic noses. *TrAC* -*Trends Anal. Chem.* **2006**, 25, 257-266.
- ¹⁵ Karlshøj, K., Nielsen, P.V., Larsen, T.O. Prediction of Penicillium expansum Spoilage and Patulin
 concentration in apples used for apple juice production by Electronic Nose Analysis. *J. Agric. Food Chem.* **2007**, 55, 4289-4298.
- ¹⁶ Jelen, H.H., Ziolkowska, A., Kaczmarek, A. Identification of the botanical origin of raw spirits produced from
 rye, potato, and corn based on volatile compounds analysis using a SPME-MS Method. *J. Agric. Food Chem.*
- **2010**, 58, 12585-12591.
- 402 ¹⁷ Marsili, R.T. SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J. Agric. Food Chem.*403 **1999**, 47, 648-654.
- 404 ¹⁸ Gardner, J.W., Philip, P.N. In *Electronic Noses: Principles and Applications*, Oxford University Press, New
 405 York, **1999**.
- 406 ¹⁹ Marcos Lorenzo, I., Pérez Pavón, J.L., Fernandez Laespada M.E., Garcia Pinto C., Moreno Cordero B.
- 407 Detection of adulterants in olive oil by headspace-mass spectrometry. J. Chromatogr. A 2002, 945, 221-30.
- 408 ²⁰ Fisk, I.D., Kettle, A., Hofmeister, S., Virdie, A., Kenny, J.S. Discrimination of roast and ground coffee aroma.
- 409 *Flavour*, **2012**, 1, 1-14.

- 410 ²¹ Sandra, P., David, F., Tienpont, B. From CGC-MS to MS-based analytical decision makers.
 411 *Chromatographia* **2004**, 60, S299–S302.
- ²² Cordero, C., Bicchi, C., Rubiolo, P. Group-Type and Fingerprint Analysis of Roasted Food Matrices (Coffee
 and Hazelnut Samples) by Comprehensive Two-Dimensional Gas Chromatography. *J. Agric. Food Chem.* **2008**, 56, 7655–7666.
- ²³ Bicchi, C., Cordero, C., Liberto, E., Sgorbini, B., Rubiolo, P. Reliability of fibres in solid-phase
 microextraction for routine analysis of the headspace of aromatic and medicinal plants. *Journal of Chromatogr. A*, **2007**, 1152, 138-149.
- 418 ²⁴ Pavon, J. L. P., Sanchez, M. Del N., Pinto, C. G.; Laespada, E. F.; Cordero, B. M., Pena, A. G. A Method for
- 419 the detection of hydrocarbon pollution in soils by Headspace Mass Spectrometry and Pattern Recognition
- 420 Techniques. Anal. Chem. 2003, 75, 2034-2041.
- ²⁵ Czerny M., Mayer F., Grosch W. Sensory study on the character impact odorants of roasted *Arabica* coffee. *J.Agric. Food Chem.*, **1999**, 47, 695–699.
- ²⁶ Blank, I.; Sen, A., Grosch, W. Potent odorants of the roasted powder and brew of *Arabica* coffee. *Z. Lebensm. Unters Forsch*, **1992**, 195, 239-245.
- 425 ²⁷ Ruosi, M.R., Liberto, E., Cordero, C., Cagliero, C., rubiolo, P., Bicchi, C., Sgorbini, B. A Further Tool To
- 426 Monitor Coffee Roasting Process: Aroma Composition And Chemical Indices. *J.Agric. Food Chem.*, in revision.
- 427 ²⁸ Determination of furan in foods, US Food and Drug Administration (FDA), Washington, DC, **2004**.
 428 www.cfsan.fda.gov/<dms/furan.html.
- 429 ²⁹ Exploratory data on furan in food, US Food and Drug Administration (FDA), Washington, DC, **2004**.
 430 www.cfsan.fda.gov/<dms/furandat.html. (27/05/2012)
- 431 ³⁰ Bicchi, C., Ruosi, M.R., Cagliero, C., Cordero, C., Liberto E., Rubiolo, P., Sgorbini, B. Quantitative analysis
- 432 of volatiles from solid matrices of vegetable origin by high concentration capacity headspace techniques:
- 433 Determination of furan in roasted coffee. J. Chromatogr. A, 2011, 1218, 753-762.

- 434 ³¹ Cozzolino, D., Smythb, H. E., Cynkara W., Janika, L., Dambergsa, R. G., Gishena, M. Use of direct
 435 headspace-mass spectrometry coupled with chemometrics to predict aroma properties in Australian Riesling
 436 wine. *Anal. Chim. Acta*, **2008**, 621, 2-7.
- 437 ³² Armanino, C., Casolino, M.C., Casale, M., Forina, M. Modelling aroma of three Italian red wines by
 438 headspace-mass spectrometry and potential functions. *Anal. Chim. Acta*, **2008**, 614, 134-142.
- ³³ Brereton R.G., In Applied Chemometrics for Scientist, Chapter 5-6, John Wiley & Sons Eds, Ltd West
 Sussex, 2007, 145-220.
- ³⁴ Berenson M.L., Levine D.M. In Statistica per le scienze economiche, Chapter 16, Zanichelli Eds, Bologna, **1993**, 549-612.
- 443 ³⁵ Wieland F., Gloess A.N., Keller M., Wetzel A., Schenker S., Yeretzian C. Online monitoring of coffee
- roasting by proton transefr reaction time-of-flight mass spectrometry (PTR-ToF-MS) towards a real-time
 process control for a consistent roast profile . *Anal. Bioanal. Chem*, **2012**, 402, 2531-2543.
- ³⁶ Wieland F., Gloess A.N., Keller M., Wetzel A., Schenker S., Yeretzian C. Highlights of analytical chemistry in
 Swizerland. *Chimia*, **2012**, 66, 443-443.
- 448 ³⁷ Romani S., Cevoli C., Fabbri A., Alessandrini L., Dalla Rosa M. Evaluation of Coffee Roasting Degree by
- Using Electronic Nose and Artificial Neural Network for Off-line Quality Control. *J. of Food Sci*, 2012, 77, 960965.
- ³⁸ Alessandrini L., Romani S., Pinnavaia G., Dalla Rosa M. Near infrared spectroscopy: An analytical tool to
 predict coffee roasting degree. *J Anal. Chim. Acta*, **2008**, 625, 95-102.

Figure captions

455 Figure 1 HS-SPME-GC-MS (a), HS-SPME-TIC-MS (b) profiles and mass spectral fingerprint (c) of an Arabica
456 coffee sample.

457

Figure 2 Scores (a) and Loading plots (b) of 55 different roasted coffee fingerprints (first 3PCs exp. var.
94.36%). Pre-processing: mean-center, full-cross validation. Categories: light roasting (solid triangle; color
57÷62), medium roasting (empty diamond; color 46÷53), dark roasting (solid diamond; colour 35÷42).
Robusta: solid line; blend 50/50: dotted line; Arabica: dashed line.

462

463 Figure 3 Regression model for furan and 2-methylfuran on 55 coffee samples *versus* the degree of roasting,
464 here represented by the experimentally-measured color.

465

466 Figure 4 OPLS regression model for color prediction as an association measure between volatile fraction,

467 spectral fingerprint, and color of 25 commercial samples, whose origins, varieties and blends are unknown. ^a

468 Correlation coefficient in Prediction, ^b Standard error in Prediction, ^c Correlation coefficient in Validation, ^d

469 Standard error in Validation

470

471



473 Figure 1 HS-SPME-GC-MS (a), HS-SPME-TIC-MS (b) profiles and mass spectral fingerprint (c) of an Arabica

474 coffee sample.

Figure 2 Scores (a) and Loading plots (b) of 55 different roasted coffee fingerprints (first 3PCs exp. var.
94.36%). Pre-processing: mean-center, full-cross validation. Categories: light roasting (solid triangle; color
57÷62), medium roasting (empty diamond; color 46÷53), dark roasting (solid diamond; colour 35÷42).
Robusta: solid line; blend 50/50: dotted line; Arabica: dashed line.





- 548 Figure 3 Regression model for furan and 2-methylfuran on 55 coffee samples *versus* the degree of roasting
- 549 here represented by the experimentally measured color.
 - Coffee samples 1.1 Normalized m/z areas 1 Ĭ 8 ğ 0000 • • • × × 0 0.9 0 ××× 000 0.8 × ŝ × × × × 0.7 ×××× X 0.6 × × 0.5 40 45 50 55 60 35 65 Color y = -0.0063x + 1.2403y = -0.0164x + 1.6413• Furan × 2-Methylfuran $R^2 = 0.8917$ $R^2 = 0.7754$

551

Figure 4 OPLS regression model for color prediction as an association measure between volatile fraction
 spectral fingerprint and color of 25 commercial samples, whose origins, varieties and blends are unknown. ^a
 Correlation coefficient in Prediction, ^b Standard error in Prediction, ^c Correlation coefficient in Validation, ^d
 Standard error in Validation



Table 1 List of samples together with varieties and blends, color values and degrees of roasting .

Sample	Varieties	Color	Roasting	Sample Varieties		Color	Roasting
Code	and blends	Values	degree	Code	and blends	Values	degree
Ara1	Arabica	61	Light	Rob13	Robusta	58	Light
Ara2	Arabica	51	Medium	Rob14	Robusta	50	Medium
Ara3	Arabica	40	Dark	Rob15	Robusta	40	Dark
Rob1	Robusta	61	Light	Ble13	Blend 50/50	60	Light
Rob2	Robusta	50	Medium	Ble14	Blend 50/50	49	Medium
Rob3	Robusta	40	Dark	Ble15	Blend 50/50	38	Dark
Ble1	Blend 50/50	59	Light	Ara16	Arabica	61	Light
Ble2	Blend 50/50	49	Medium	Ara17	Arabica	51	Medium
Ble3	Blend 50/50	39	Dark	Ara18	Arabica	42	Dark
Ara4	Arabica	59	Light	Rob16	Robusta	62	Light
Ara5	Arabica	49	Medium	Rob17	Robusta	50	Medium
Ara6	Arabica	40	Dark	Rob18	Robusta	40	Dark
Rob4	Robusta	60	Light	Ble16	Blend 50/50	62	Light
Rob5	Robusta	50	Medium	Ble17	Blend 50/50	49	Medium
Rob6	Robusta	41	Dark	Ble18	Blend 50/50	39	Dark
Ble4	Blend 50/50	61	Light	C1	Commercial	57	Light
Ble5	Blend 50/50	51	Medium	C2	Commercial	53	Medium
Ble6	Blend 50/50	42	Dark	C3	Commercial	47	Medium
Ara7	Arabica	62	Light	C4	Commercial	53	Medium
Ara8	Arabica	51	Medium	C5	Commercial	58	Light
Ara9	Arabica	39	Dark	C6	Commercial	39	Dark
Rob7	Robusta	62	Light	C7	Commercial	56	Light
Rob8	Robusta	49	Medium	C8	Commercial	58	Light
Rob9	Robusta	40	Dark	C9	Commercial	43	Dark
Ble7	Blend 50/50	60	Light	C10	Commercial	48	Medium
Ble8	Blend 50/50	51	Medium	C11	Commercial	39	Dark
Ble9	Blend 50/50	40	Dark	C12	Commercial	52	Medium
Ara10	Arabica	62	Light	C13	Commercial	50	Medium
Ara11	Arabica	50	Medium	C14	Commercial	43	Dark
Ara12	Arabica	41	Dark	C15	Commercial	48	Medium
Rob10	Robusta	59	Light	C16	Commercial	61	Light
Rob11	Robusta	51	Medium	C17	Commercial	52	Medium
Rob12	Robusta	38	Dark	C18	Commercial	47	Medium
Ble10	Blend 50/50	58	Light	C19	Commercial	56	Light
Ble11	Blend 50/50	49	Medium	C20	Commercial	40	Dark
Ble12	Blend 50/50	38	Dark	C21	Commercial	47	Medium
Ara13	Arabica	59	Light	C22	Commercial	57	Light
Ara14	Arabica	49	Medium	C23	Commercial	48	Medium
Ara15	Arabica	40	Dark	C24	Commercial	46	Medium
				C25	Commercial	52	Medium

569 Table 2 List of markers identified in HS-SPME-GC-MS profile of an Arabica roasted sample together with their

570 target (TI) and qualifier (Qi) ions. *Markers tentatively identified through their MS-EI fragmentation patterns

ID	Compounds	Ret Time	Ι ^τ cw	I ^T OV1	ті	Qi1	Qi2
1	Furan	3 74	837	500	68	58	30
2	2-methylfuran	4 49	873	586	82	81	53
3	2-methyl butanal	5.09	903	641	86	57	41
4	3-methyl butanal	5.09	904	635	86	71	57
5	2 5-dimethylfuran	5.86	938	691	96	95	81
6	2.3-butanedione	6.31	960	555	86	57	43
7	2.3-pentandione	8 4 9	1043	668	100	57	43
8	2-vinvlfuran*	9.00	1059	-	94	65	66
9	2.3-bexanedione	10.88	1117	756	43	71	43
10	1-methyl-pyrrole	11 18	1124	715	81	80	66
11	2-vinvl-5-methylfuran*	11 79	1139	-	108	107	79
12	Pyridine	12 62	1165	720	79	52	39
13	Pyrazine	13.85	1195	709	80	53	70
14	methylpyrazine	16.08	1249	802	94	67	53
15	3-hvdroxy-2-butanone	16.84	1265	681	88	73	45
16	1-hydroxy-2-propanone	17 42	1278	626	74	43	41
17	2 5-dimethylpyrazine	18 45	1306	893	108	81	42
18	2 6-dimethylpyrazine	18 74	1313	889	108	81	42
19	ethylpyrazine	19.01	1318	895	107	108	80
20	2.3-dimethylpyrazine	19 45	1329	904	108	67	93
21	1-hvdroxy-2-butanone	20.57	1353	732	88	57	42
22	3-ethyl-pyridine	20.85	1364	934	107	92	79
23	2-ethyl-6-methylpyrazine	21.23	1371	977	121	122	94
24	2-ethyl-5-methylpyrazine	21.46	1376	981	121	122	94
25	trimethylpyrazine	21.96	1389	984	122	81	42
26	2-ethyl-3-methylpyrazine	22.01	1391	985	121	122	80
27	2-propylpyrazine	22.60	1402	985	94	107	122
28	2-ethyl-3.6-dimethyl pyrazine	23.78	1427	1059	135	136	108
29	acetic acid	23.79	1432	547	60	43	45
30	furfural	24.45	1443	801	96	95	39
31	1-acetoxy-2-propanone	24.57	1448	825	43	86	116
32	2-acetylfuran	26.12	1483	882	95	110	39
33	furfuryl acetate	27.64	1521	963	81	98	140
34	5-methyl furfural	28.86	1551	933	110	109	81
35	1-methyl-2-carboxaldehyde pyrrole	30.67	1596	974	109	108	80
36	furfuryl alcohol	32.37	1640	823	98	81	69
37	1-furfuryl pyrrole	38.58	1805	1152	81	147	53
38	guaiacol	39.54	1832	1064	109	124	81
39	2-acetyl pyrrole	43.27	1941	1030	94	109	66
40	2-carboxaldehyde pyrrole	44.98	1991	976	95	94	66
41	p-vinylguaiacol	50.47	2163	1289	150	135	107

571

- 573 Table S1 One-way ANOVA results on Total Volatile fraction spectral fingerprint areas of coffee pod samples
- 574 (n=5; α=0.05).

Groups	Count	Sum	Mean	Variance
Fibre 1 (F1)	5	103.41	20.68	2.38
Fibre 2 (F2)	5	134.87	26.97	0.22
Fibre 3 (F3)	5	103.14	20.63	0.52

One-Way Anova

Source of variation	SQ	gdl	MQ	F	Significant Value	F crit
Between groups	133.08	2	66.54	63.86	4.01E-07	3.89
Within groups	12.50	12	1.04			
Total	145.59	14				

575

577 Table S2 Method precision (repeatability and intermediate precision) of total fingerprint and some 578 characteristic ions expressed as Relative Standard Deviation % calculated over replicates collected across the 579 entire period (12 months). * m/z ions characterizing key-aroma compounds according to Czerny²³ and Blank²⁴.

580

m/z	Characterizing	Repeatability	Intermediate
37	Roasting	1 30	14 15
38	Roasting	1.00	9 32
39	Aroma and roasting	0.38	5.06
41	Aroma and roasting	0.84	16 27
45	Aroma and varieties	2 25	4 65
50	Aroma and roasting	0.36	9 29
51	Aroma and roasting	1.06	8.04
52	Aroma and roasting	1.00	8 50
52	Aroma and roasting	2 27	6 50
60	Aroma and varieties	1 70	8.00
62	Roasting	1.70	8.00
66	Roasting and varieties	1.05	9.28
67	Roasting and varieties	0.00	8.48
68	Tovic	1 02	7.8/
60	Aroma and roasting	1.72	10 37
70	Aroma and roasting	2 10	22 77
70	Roasting	1.67	0.20
70	Roasting	1.07	12.67
80	Roasting	0.58	10.35
81	Roasting and varieties	1.60	9 10
82	Tovic	0.58	6 50
86*	Δroma	2.62	7.62
88	Aroma and varieties	2.02 1.62	16 12
Q/	Aroma and roasting	1 1/	13 56
95	Roasting and varieties	1.14	7.68
96	Roasting and varieties	2.27	0.82
07	Roasting	2.22	5.26
98	Roasting	2.27	7.67
100*	Δroma	2.31 A A7	13 7/
100	Aroma and roasting	0.50	11 06
100*	Aroma and roasting	1 3/	6.98
107	Roasting	1.54	7.86
10	Aroma and roasting	2/0	7.00 Q QQ
127	Aroma and roasting	2.77	11 32
125*	Aroma	2.54	5 10
150*	Aroma	2.44 11.24	20.08
Total			_0.00
Fingerprint		1.27	3.16
Area			

581

- Table 3 Indices (m/z ratios) and related correlation equation and coefficients with the color for each variety
 and/or blend analyzed together with each index value and its standard deviation.

Index m/z ratios	Variety or blend	Equation	r2	Index value	Index Standard deviation	Mean color value
	100% ARABICA		0.8754	1051	226	60
		y = -119x + 8018		1765	358	50
		5		3426	600	40
70/110				1161	207	60
79/110	BLEND	y = -110x + 7604	0.9872	1796	310	50
		5		3361	343	40
				1148	111	60
	100% ROBUSTA	y = -63x + 4874	0.9550	1621	135	50
		5		2407	123	40
	100% ARABICA		0.8946	879	76	60
		y=-51x+3884		1239	143	50
		5		1898	139	40
07/110	BLEND	y=-48x+3872	0.9912	1013	101	60
97/110				1382	154	50
				1979	139	40
	100% ROBUSTA		0.9925	1148	118	60
		y=-41x+3571		1529	90	50
				1958	107	40
			0.8896	1692	172	60
	100% ARABICA	y=-108x+8043		2438	303	50
				3846	325	40
				1960	206	60
98/110	BLEND	y=-101x+7913	0.9918	2725	333	50
				3971	285	40
				2203	252	60
	100% ROBUSTA	y=-81x+7034	0.9939	2967	171	50
				3818	229	40

sample	Measured Y	Predicted Y	Residual Y	Upper Limit	Lower Limit
Ara2	51	52	-0.71	57	46
Roh3	40	<u>الح</u>	-4 59	50	30
Rio1	40 50	40 56	-4.55	50 61	55
Ara5	10	40	2.75	54	31
RIAJ	45 61	45	0.02	61	44 51
Die4 Dob7	62	50	4.02	62	52
Rub7 Rob8	40		J. 13 1 12	02 52	12
	49	40 50	1.12	55 65	43 E4
Alaiu Dah10	6Z	59 E4	Z.74 5.40	00 50	04 40
RUDIU Dia10	59	54 20	D. 10	59	49
Bie12	38	38	-0.43	44	33
Ara15	40	40	0.32	45	34
Ble13	60	57	3.21	62	52
Ble14	49	48	0.80	53	43
Ara18	42	42	0.08	47	37
Rob18	40	43	-2.90	48	38
Ble16	62	58	4.25	63	53
C19	56	62	-5.84	67	57
C21	47	57	-9.79	62	52
C24	46	55	-9.26	60	50
C14	43	36	6.66	42	31
C17	52	52	-0.43	58	47
C1	57	56	1.27	61	50
C4	53	54	-0.91	59	49
C6	39	41	-2.05	47	36
C10	48	55	-6.76	60	50
C12	52	53	-1.22	58	48
Prediction Equation	rpred	SEP	Model validation error on 2	rval	SEV
y=0.754x+12.71	0.8416	4.29	Factors	0.9399	2.68

588 Table 4 Color measured with the three indices through PLS elaboration and model parameters