JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Real-Time Monitoring of 4-Vinylguaiacol, Guaiacol, and Phenol during Coffee Roasting by Resonant Laser Ionization Time-of-Flight Mass Spectrometry

RALPH DORFNER,† THOMAS FERGE,† ANTONIUS KETTRUP,† RALF ZIMMERMANN,*,† AND CHAHAN YERETZIAN*,‡

GSF, National Research Center for Environment and Health, Institute of Ecological Chemistry, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany, and Nestlé Research Center, Vers-chez-les-Blanc, P.O. Box 44, CH-1000 Lausanne, Switzerland

The formation of 4-vinylguaiacol, guaiacol, and phenol during coffee roasting was monitored in real-time, using resonance enhanced multiphoton ionization and time-of-flight mass spectrometry. A model is proposed, based on two connected reaction channels. One channel, termed the "low activation energy" channel, consists of ester hydrolysis of 5-FQA followed by decarboxylation of the ferulic acid to form 4-vinylguaiacol, and finally polymerization at the vinyl group to form partly insoluble polymers (coffee melanoidins). The second "high activation energy" channel opens up once the beans have reached higher temperatures. It leads to formation of guaiacol, via oxidation of 4-vinylguaiacol, and subsequently to phenol and other phenolic VOCs. This work aims at developing strategies to modify the composition of coffee flavor compounds based on the time−temperature history during roasting.

KEYWORDS: Coffee; aroma; roasting; real-time analysis; mass spectrometry; resonant laser ionization

INTRODUCTION

Coffee is one of the most popular beverages in the world, owing mainly to its unique flavor. Among the many processes that coffee undergoes, from the seed to the cup, roasting is undoubtedly crucial. A green coffee bean contains all necessary ingredients for the development of its flavor. Yet, coffee has to be roasted to unlock its flavor.

Scientific effort to elucidate the origin of the rich and distinctive coffee flavor can be traced back to the 19th Century when Bernheimer identified in 1880 the first few volatile organic compounds (VOCs) of coffee (*1*−*3*). Today close to 900 VOCs have been reported in coffee (*4*). Among these, only a small fraction, approximately 5%, are odoriferous, and less than 30 are believed to be important flavor compounds of roasted coffee (*5*−*13*).

Recently, we embarked on a real-time study of VOCs released during coffee roasting as a means to elucidate formation mechanisms of coffee flavor compounds. Among the various methods tested, we have chosen to pursue two, which we consider to be the most promising. Both are based on direct injection mass spectrometry using soft ionization techniques. One approach uses lasers for resonant multiphoton ionization (*14*, *15*). The other is based on chemical ionization, termed proton transfer reaction-mass spectrometry (PTR-MS) (*16*−*20*). In recent papers, we have outlined the advantages and limitations of both approaches (*21*−*23*).

Here we report on a study of the formation mechanisms of three phenolic coffee flavor compounds—4-vinylguiacol, guaiacol, and phenol—whose formation pathways are believed to be linked (degradation of chlorogenic acids), using resonance enhanced multiphoton ionization (REMPI). 4-Vinylguiacol and guaiacol have phenolic/spicy/smoky sensory notes and are key flavor compounds of coffee. Phenol has a medicinal odor and does not contribute to the flavor of coffee.

Kinetic data on the evolution of odorous VOCs during roasting are rare, and mainly based on off-line GC analysis of samples taken at different times during roasting (*24*−*28*). Where possible, we relate our results to these former studies.

MATERIALS AND METHODS

Coffee Roasting. Eighty grams of Arabica coffee beans (Colombia) was roasted for 600 s at 225 °C on a laboratory-scale commercial roaster that has two identical and independent rotating drums (*Probat* BRZ 2; see **Figure 1**). This led to a very dark roast. The roaster off-gas was analyzed in real-time by REMPI-TOFMS@266 nm (see below).

Roast Degree. Roast degree was determined based on measured total weight loss (*29*−*31*). Coffee beans were roasted for a given time, rapidly air-cooled, and the total weight loss was determined. Light roast corresponds to 13−16% total weight loss, medium roast corresponds to 16−19%, dark roast corresponds to 19−23%, and a very dark roast corresponds to >23%. This procedure allowed calibrating the time axis with respect to roast level. As a check for the stability of the roaster operations, and of the calibration of the time axis, we also monitored the temperature inside the roaster and evaluated the color of the beans.

* Corresponding authors: chahan.yeretzian@rdls.nestle.com; tel: +41-(0)21-785.86-15; ralf.zimmermann@gsf.de;tel.: +49-(0)821-59 83 005.
† GSF, National Research Center for Environment and Health.
‡ Nestlé Research Center.

10.1021/jf0341767 CCC: $25.00
Published on Web 08/08/2003

Figure 1. Photographs of the laboratory scale coffee roaster with sampling unit.

A thermocouple mounted inside the roaster and in contact with the coffee beans monitored the temperature profile during each roasting experiments. This was used to check for consistency of the roaster relative to the original calibration experiment. In addition, coffee roasted to different roast degrees (known roasting time/total weight loss) were used as color references, to ensure the consistency of the calibration of the roast level.

Resonance Enhanced Multiphoton Ionization. Selective and time-resolved monitoring of VOCs in the roaster off-gas was achieved by REMPI at 266 nm, and time-of-flight mass spectrometry (TOFMS). Selectivity was introduced into the ionization step by resonant ionization at a fixed ultraviolet (UV) laser wavelength. Depending on molecular resonances, VOCs with an optical (electronic) absorption at 266 nm absorb a laser photon, while those transparent at 266 nm remain in the ground state. The width of optical absorptions is given by the ground-state population, and broadens by the molecule's temperature, which itself depends on the expansion conditions at the inlet system (*15*). Since we were using an effusive molecular beam (no cooling), a range of rotational and vibrational states were populated, resulting in broad absorption bands. Consequently, a range of compounds may be ionized simultaneously, due to overlapping absorption bands (*14*).

In a typical REMPI ionization scheme, molecules absorb a first photon and are excited into a UV electronic state. These excited molecules are subsequently ionized by absorbing a second photon. For effective and selective REMPI detection, the following conditions have to be fulfilled: (i) *resonance condition:* the molecule has a UV-active excited state, whose energy corresponds to the energy of the laser photon, (ii) *lifetime condition:* the excited state has a lifetime which is long enough for it to absorb a second photon for ionization, and (iii) *ionization condition:* the energy of two photons is equal or higher than the ionization energy of the molecule.

The home-built mobile device that was used consisted of a Reflectron-TOFMS analyzer, an effusive beam inlet system and a built-in laser operated at 266 nm (Continuum Nd:YAG laser SURELIGHT, 266 nm) (*32*). Data acquisition and analysis were performed via a 250 MHz transient recorder unit (Acqiris, DP110 on PC) at an acquisition rate of 10 Hz, using LabVIEW.

Real-Time VOC Sampling. Figure 2 gives a schematic overview of the experimental setup, to illustrate the sampling of the roaster gas and the introduction of the volatiles into the TOFMS. A quartz tube with a passivated inner surface of 10-mm inner diameter was used to sample gas from the roaster. The tube reached about 2 cm into the rotating drum. A constant off-gas sampling stream of 1.5 L/min was pumped through the sampling system. A quartz wool paper filter was put inside the tube to prevent solid contamination such as dust or silver skins reaching the capillary inlet system. All sampling lines were heated to 250 °C, to minimize condensation of low-volatile compounds.

RESULTS

Figure 3 shows a typical REMPI@266 nm mass spectrum while roasting 80 g of Arabica coffee at 225 °C. The laser power density was adjusted to 10^6-10^7 W/cm^2 to avoid nonresonant ionization processes. The spectrum contains predominantly molecular ions. As discussed in a previous paper (*23*), chemical assignment of the ion peaks was based on three distinct pieces of information: the literature on coffee flavor compounds (*4*), the mass as observed in TOFMS, and optical absorption properties. Using this information, many volatiles observed in **Figure 3** were unambiguously identified.

In this study, we concentrated on three phenolic VOCs, two of which are known to contribute to coffee flavor. These are 4-vinylguaiacol (150 *m*/*z*), guaiacol (124 *m*/*z*), and phenol (94 *m*/*z*). Their time−intensity profiles during roasting are shown in **Figure 4**. Using a laser repetition rate of 10 Hz, and numerically integrating five laser-shots for each data point, we obtained a time resolution of 0.5 s.

On the basis of weight loss measurements, the following calibration of the time axis in terms of roast degree (roasting at 225 °C) was obtained: 200 s after beginning of roasting, the beans reached approximately 100 °C, a temperature they maintained until about 300 s. During this initial stage of roasting, the coffee beans were mainly drying. The 10% moisture content of the green beans was reduced to about 5% before the bean temperature increased beyond 120 °C. We observed that this occurs at about 300 s. After an additional 20 s (approximately

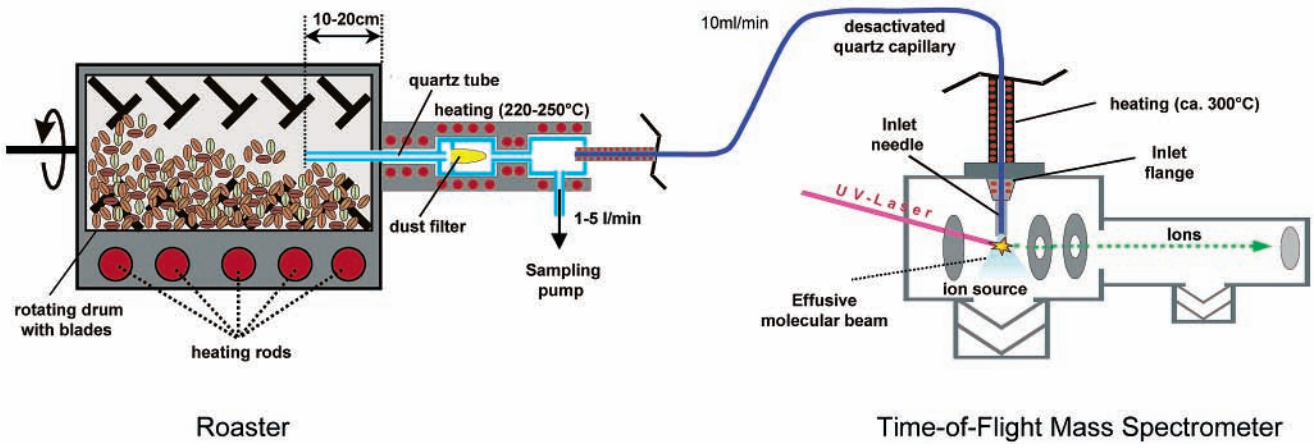

Figure 2. Schematic representation of the experimental setup including the laboratory scale coffee roaster with sampling unit and laser mass spectrometer.

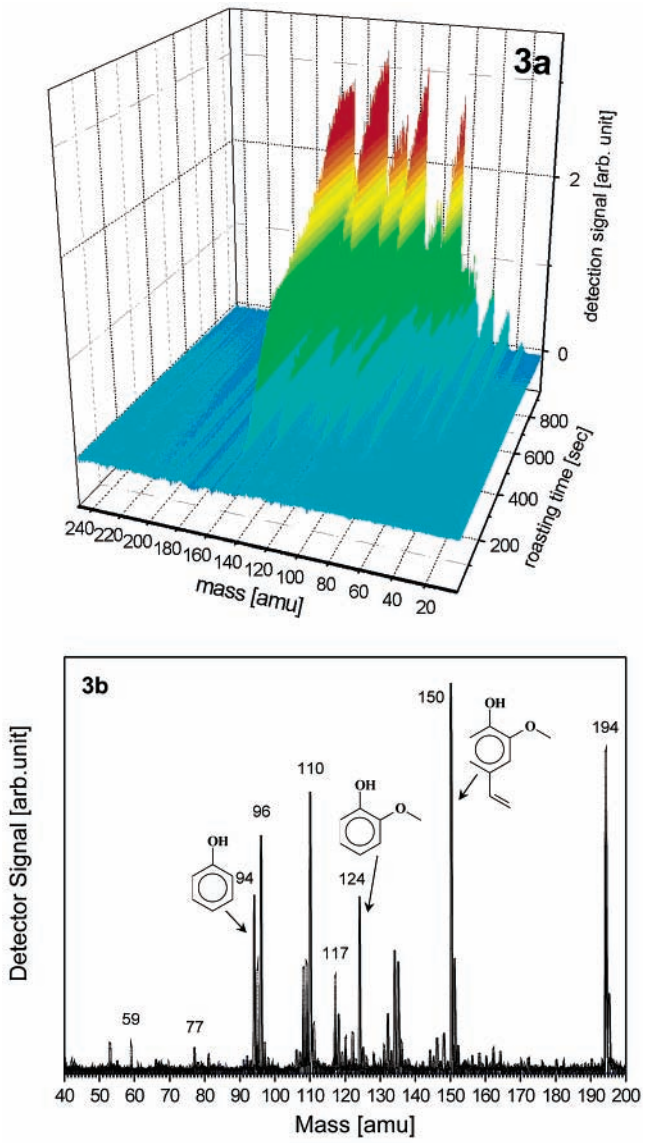

Figure 3. Real-time REMPI@266 nm–TOFMS of roast-gas while roasting 80 g of Arabica coffee. Panel a represents the full time/mass/intensity 3D plot as it is recorded during roasting. Panel b shows a time/intensity cross-section from panel a at a fixed time (medium roast level). The three phenolic VOCs, phenol (94 *m*/*z*), guaiacol (124 *m*/*z*), and 4-vinylguaiacol (150 *m*/*z*), are efficiently ionized at 266 nm. Their formation is discussed in more detail in the text. In addition, furfurylalcohol (96 *m*/*z*), dihydroxybenzene (110 *m*/*z*), indol (117*m*/*z*), and caffeine (194 *m*/*z*) are also seen, to name a few of th e other prominent ion peaks.

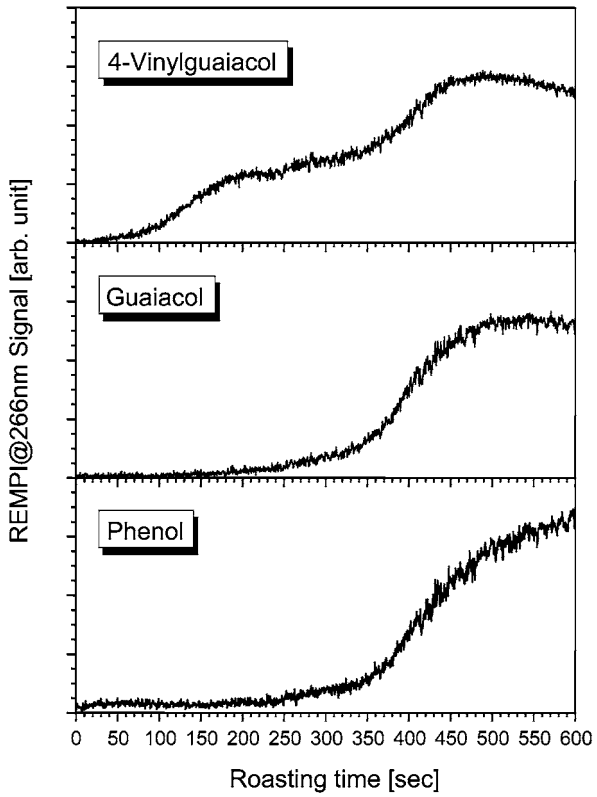

Figure 4. REMPI@266 nm–TOFMS time–intensity profiles of 4-vinylguaiacol (top trace), guaiacol (middle trace), and phenol (bottom trace) while roasting at 225 °C.

170 °C bean temperature), pyrolysis reactions started, and the coffee roasting process became exothermic. A light roast was reached at approximately 360 s. Continuing the roasting, a medium roast was obtained at 400 s and a dark roast at 450 s. Beyond this point, beans became over-roasted. In the experiment shown in **Figure 4**, roasting was stopped after 600 s.

DISCUSSION

Depending on the variety, green coffee beans may contain up to 10% (dry-weight) chlorogenic acids (CQA) (*33*). Robusta coffee is known to be richer in CQA than Arabica coffee (7−10% in Robusta; 5−7.5% in Arabica). During roasting most of the CQAs are degraded, generating mainly nonvolatile compounds known as melanoidins, while a small fraction appears as various phenolic VOCs in coffee (approximately 1%) (*33*). Robusta coffee was reported to have a relatively higher content of phenolic VOCs than Arabica (*8*, *9*, *34*−*37*) in accordance with its higher CQA content. This also fits with REMPI@266 nm headspace spectra on Arabica and Robusta brews, in which higher phenolic VOCs were observed in Robusta coffee (*38*).

Various groups have investigated the fate of CQA during roasting (*39*−*44*). In particular, Tressl et al. performed model experiments, indicating that thermal degradation of 5-feruloylquinic acid (5-FQA) via the intermediate ferulic acid is the main precursor of 4-vinylguaiacol and guaiacol (*42*). They proposed a degradation pathway summarized in **Figure 5**. Wynnes et al. detected feruloylquinic acid lactone in coffee, indicating that 5-FQA reacts via intramolecule esterification (*45*). Henrich and Bates pointed out that only a relatively small amount of 4-vinyguaiacol is found in coffee compared to the amount of 5-FQA that is consumed (*46*, *47*). Furthermore, it has been observed that the ratio of guaiacol to 4-vinylguaiacol is larger in dark roasted coffee than in a light or medium roast coffee (*42*). Combining these findings and the results reported here, the following interpretation for the formation mechanism of 4-vinylguaiacol and guaiacol is proposed.

Formation of 4-vinylguaiacol (**Figure 4**) occurs rapidly already at the very early stages of roasting (during the first 200 s), where the bean temperature is believed to increase steadily from room temperature to about 100 °C. Once the beans have reached 100 °C, most of the heat energy is absorbed by the water in the beans, transferring heat into evaporation of water. This continues for some 100 s, and momentarily prevents a further increase of the bean temperature. During this drying phase, the rate of 4-vinylguaiacol formation increased only a little. Once the beans have lost most of their free water (300 s), the bean temperature rises again, driving the rate of 4-vinylguaiacol formation to even higher values.

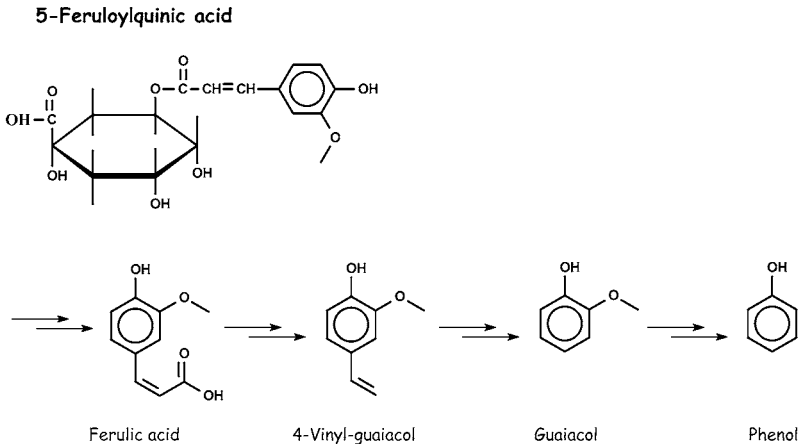

Figure 5. Proposed degradation pathway of 5-feruloylquinic acid, based on results from model experiments of Tressl et al. (*42*).

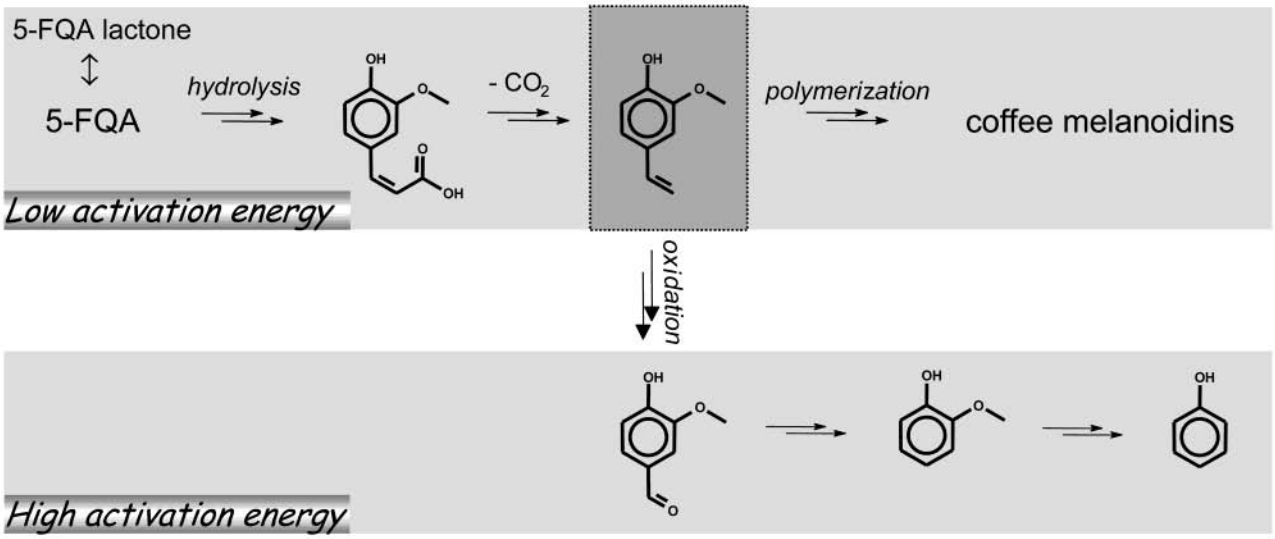

Figure 6. Two-channel model for the degradation of 5-FQA and the concomitant formation of melanoidins and phenolic VOCs during coffee roasting.

The mechanism in **Figure 5** suggests that ferulic acid is an intermediate in the reaction from 5-FQA to 4-vinylguaiacol. Measurements of loss of 5-FQA by Leloup et al. have shown that more than 50% of 5-FQA is lost before even reaching the exothermic phase, and around 80% have disappeared at the light roast stage. Considering that degradation of 5-FQA proceeds via hydrolysis, the high rate of degradation in the first phase of roasting might be related to the relatively high moisture content of the beans. Leloup et al. detected only minute amounts of free ferulic acid during roasting. Either ferulic acid is present in some derivatized (esterified) form (*44*) or decarboxylation of the carboxylic group proceeds instantaneously to form 4-vinylguaiacol. 4-Vinylguaiacol formation gradually slows down at longer roasting times and starts to fall off beyond 500 s.

Referring to **Figure 4**, significant formation of guaiacol occurs once the roasting process has entered the exothermic phase. Since its precursor 4-vinylguaiacol (**Figure 5**) is readily generated much earlier during roasting, this late formation of guaiacol indicates that higher temperatures are required for the reaction 4-vinylguaiacol → guaiacol. This is consistent with the fact that oxidation of 4-vinylguaiacol to form guaiacol is a process that requires higher energies (relative to hydrolysis of 5-FQA and decaboxylation of ferulic acid).

We initially observe a very slow formation of guaiacol during the drying phase (0−300 s), although large amounts of 4-vinylguaiacol have already been generated. Considering that the unsaturated vinyl-unit of 4-vinylguaiacol may react via intermolecular polymerization, it is likely that during the first phase of roasting 4-vinylguaiacol contributes to the formation of coffee melanoidins. Once the beans have dried sufficiently, the temperature rises again, triggering the formation of guaiacol. This happens after about 350 s, when the temperature of the beans reaches about 170 °C. The concentration of guaiacol in the roast gas is seen to increase strongly from a light to a dark roast, consistent with the literature (*25*, *35*, *48*). At higher roasting times, when the coffee becomes over-roasted, the formation rate of guaiacol decreases again, yet later and less pronounced than for 4-vinylguaiacol.

Finally, we see phenol appear in the roast gas. First, it is following the time−intensity curve of guaiacol. Yet, at 500 s, where guaiacol concentrations start to decrease, the formation rate of phenol increases until the end of roasting (over-roasted beans), in accordance with former reports (*24*, *26*, *48*). This is consistent with the fact that besides the pathway depicted in **Figure 5**, a multitude of additional degradation pathways may feed into the phenol channel.

Summarizing the above discussion, we propose the following reaction scheme: Degradation of 5-FQA, which is partially derivatized to lactone by intramolecular esterification, proceeds via two connected reaction channels, see **Figure 6**. We label the first the "low activation energies" channel (endothermic

phase). It dominates in the early stages of roasting, where the bean temperature is relatively low (below 120 °C) and the moisture content is high. It consists of ester hydrolysis of 5-FQA followed by decarboxylation of the ferulic acid to form 4-vinylguaiacol, and finally polymerization at the vinyl group to form partly insoluble polymers (melanoidins). Whether some transitory intramolecular esterification (lactone formation) on the 5-FQA or some esterification of the ferulic acid unit occurs is unclear. Yet, as long as the temperature is below about 170 °C, we believe that the scheme shown in the top part of **Figure 6** represents the main sequence of reactions.

Once the beans have dried, the bean temperature rises. On the one hand, this increases the rate of 4-vinylguaiacol formation (and probably of its polymerization). On the other hand, a new "high activation energy" channel opens up, shown in the bottom part of **Figure 6**, which sequentially leads to guaiacol and phenol. Referring to Leloup et al. (*44*), essentially all 5-FQA has disappeared at the very dark roast stage. Eventually, the formation of guaiacol slows down, as 5-FQA is used up.

CONCLUSION

Monitoring in real-time the time−intensity evolution of three phenolic VOCs of coffee while roasting, a model is proposed for their formation pathway. It consists of two channels of reaction sequences, one that is activated at lower bean temperatures (endothermic phase) and one that opens up when higher temperatures are reached (exothermic phase).

Along the first "low activation energy" channel, 5-FQA hydrolyzes to form ferulic acid, which in turn decarboxylates to 4-vinylguaiacol, and finally polymerizes at the vinyl group to form partly insoluble polymers (melanoidins). For roasting temperatures below about 150 °C, this is proposed to be the main sequence of reactions.

Once the beans have dried, the bean temperature rises. This increases the rate of 4-vinylguaiacol formation, and opens up a new "high activation energy" channel, which leads sequentially to guaiacol, via oxidation of 4-vinylguaiacol, and subsequently to phenol (and other phenolic VOCs).

In the framework of the proposed model, the partitioning of the CQA degradation pathways between polymerization from 4-vinylguaiacol and formation of guaiacol could be achieved by controlling the time−temperature profile during roasting. In more general terms, the results and the model presented here indicate that control of the time−temperature history during roasting has implications for the composition of the soluble and nonsoluble melanoidin fraction, as well as for the flavor profile of phenolic flavor compounds.

ACKNOWLEDGMENT

We acknowledge Karin Kraehenbuehl for stimulating discussions, and thank Elizabeth Prior for critical proofreading of the manuscript.

LITERATURE CITED

(1) Grosch, W. *Chem. Zeit* **1996**, *30*, 126−33.
(2) Parliment, T. H.; Stahl, H. D. *Chemtech* **1995**, *August*, 38−47.
(3) Bernheimer, O. *Monatsh. Chem.* **1880**, *1*, 456−67.
(4) *Volatile Compounds in Food*, 7th ed.; TNO Nutrition and Food Research Institute: Zeist, The Netherlands, 1996.
(5) Blank, I.; Sen, A.; Grosch, W. *ASIC-14eme Colloque Scientifique International sur le Café*, 1992 7−14−1991; ASIC; 117−29.
(6) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. *J. Agric. Food Chem.* **1991**, *39*, 757−59.
(7) Blank, I.; Sen, A.; Grosch, W. *Z. Lebensm.-Unters. Forsch.* **1992**, *195*, 239−45.
(8) Semmelroch, P.; Grosch, W. *J. Agric. Food Chem.* **1996**, *44*, 537−43.
(9) Semmelroch, P.; Grosch, W. *Lebensm.-Wiss. Technol.* **1995**, *28*, 310−13.
(10) Czerny, M.; Mayer, F.; Grosch, W. *J. Agric. Food Chem.* **1999**, *47*, 695−99.
(11) Grosch, W.; Mayer, F. *ACS Symp. Ser. 763* **2000** ACS, 430−38.
(12) Grosch, W.; Czerny, M.; Mayer, F.; Moors, A. *ACS Symp. Ser. 754* **2000** ACS, 202−09.
(13) Grosch, W. *Coffee: Recent Developments*; Clarke, R. J., Vitzthum, O. G., Eds.; Blackwell Science: London, 2001; Chapter 3.
(14) Zimmermann, R.; Heger, H. J.; Yeretzian, C.; Nagel, H.; Boesl, U. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1975−79.
(15) Dorfner, R.; Zimmermann, R.; Yeretzian, C.; Kettrup, A. *ASIC-18eme Colloque Scientifique International sur le Café*, 2000, 1999; ASIC: 136−42.
(16) Yeretzian, C.; Jordan, A.; Brevard, H.; Lindinger, W. *ACS Symp. Ser. 763* **2000** ACS; 58−72.
(17) Yeretzian, C.; Jordan, A.; Badoud, R.; Lindinger, W. *Eur. Food Res. Technol.* **2002**, *214*, 92−104.
(18) Hansel, A.; Jordan, A.; Holzinger, R.; Prazeller, P.; Vogel, W.; Lindinger, W. *Int. J. Mass Spec. Ion Phy.* **1995**, *149/150*, 609−19.
(19) Lindinger, W.; Hansel, A.; Jordan, A. *Chem. Soc. Rev.* **1998**, *27*, 347−54.
(20) Lindinger, W.; Hansel, A.; Jordan, A. *Int. J. Mass Spec.* **1998**, *173*, 191−241.
(21) Dorfner, R.; Zimmermann, R.; Kettrup, A.; Yeretzian, C.; Jordan, A.; Lindinger, W. *Lebensmittelchemie* **1999**, *53*, 32−34.
(22) Fay, L. B.; Yeretzian, C.; Blank, I. *Chimia* **2001**, *55*, 429−34.
(23) Dorfner, R.; Ferge, T.; Uchimura, T.; Yeretzian, C.; Zimmermann, R.; Kettrup, A. *ASIC-19eme Colloque Scientifique International sur le Café*, 2002 2001; ASIC.
(24) Gianturco, M. A. Coffee Flavor. *The Chemistry and Physiology of flavor*; AVI Publishing Company, Inc.: Westport, CT, 1967; pp 431−49.
(25) Silwar, R.; Lüllmann, C. *Café Cacao Thé* **1993**, *37*, 145−52.
(26) Schenker, S.; Perren, R.; Escher, F.; Heinemann, C.; Huber, M.; Pompizzo, R. *J. Food Sci.* **2002**, *67*, 60−66.
(27) Kawakami, Y.; Kuneida, A.; Sato, Y.; Takashima, T.; Kanisawa, T. *ASIC-16eme Colloque Scientifique International sur le Café*, 1995 4−9−1995; ASIC.; 332−39.
(28) Hashim, L.; Chaveron, H. *Food Res. Int.* **1996**, *28*, 619−23.
(29) Sivetz, M.; Desrosier; N. W. *Coffee Technology*; AVI Publishing Co.: Westport, CT, 1979; Chapter 8.
(30) Clarke, R. J.; Macrae, R. *Coffee, Volume 2: Technology*; Elsevier Applied Science Publishers: 1987.
(31) Illy, A.; Viani, R. *Espresso Coffee, The Chemistry of Quality*; Academic Press Limited: London, 1995.
(32) Heger, H. J.; Zimmermann, R.; Dorfner, R.; Beckmann, M.; Griebel, H.; Kettrup, A.; Boesl, U. *Anal. Chem.* **1999**, *71*, 46−57.
(33) Clarke, R. J.; Macrae, R. *Coffee, Volume 1: Chemistry*; Elsevier Applied Science Publishers: New York, 1986.
(34) Balyaya, K. J.; Clifford, M. N. *ASIC-16eme Colloque Scientifique International sur le Café*, 1995 4−9−1995; ASIC; 316−25.
(35) Mayer, F.; Czerny, M.; Grosch, W. *Eur. Food Res. Technol.* **1999**, *209*, 242−50.
(36) Mayer, F.; Czerny, M.; Grosch, W. *Eur. Food Res. Technol.* **2000**, *211*, 272−76.

(37) Belitz, H. D.; Grosch, W.; Schieberle, P. *Lehrbuch der Lebensmittelchemie*, 5.; vollst. überarb. Aufl. ed.; Springer-Verlag: Berlin, 2001.
(38) Zimmermann, R.; Heger, H. J.; Dorfner, R.; Yeretzian, C.; Kettrup, A.; Boesl, U. *Food Ingredients: New Technologies − Fruits & Vegetables*; UNIDO (United Nations Industrial Development Organisations): 1997; 343−50.
(39) Purdon, M. P.; McCamey, D. A. *J. Food Sci.* **1987**, *52*, 1680−83.
(40) Scholz-Böttcher, B. M.; Maier, H. G. *ASIC-14eme Colloque Scientifique International sur le Café*, 1992 1991; ASIC; 220−29.
(41) König, W. A.; Sturm, R. *ASIC-10eme Colloque Scientifique International sur le Café*, 1983 1982; ASIC; 271−78.
(42) Tressl, R. *Thermal Generation of Aromas*; Parliment, T. H.; McGorrin, R. J., Ho, C.-T., Eds.; American Chemical Society: Washington, DC, 1989; Chapter 27.
(43) Trugo, L. C.; Macrae, R. *Food Chem.* **1984**, *15*, 219−27.
(44) Leloup, V.; Louvrier, A.; Liardon, R. *ASIC-16eme Colloque Scientifique International sur le Café*, 1995 4−9−1995; ASIC; 192−98.
(45) Wynne, K. N.; Familari, M.; Boublik, J. H.; Drummer, O. H.; Rae, I. D.; Funder, J. W. *Clin. Exp. Pharm. Physiol.* **1987**, *14*, 785−90.
(46) Heinrich, L.; Baltes, W. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 366−70.
(47) Heinrich, L.; Baltes, W. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 362−65.
(48) Gretsch, C.; Sarrazin, C.; Liardon, R. *ASIC-18eme Colloque Scientifique International sur le Café*, 2000 8−2−1999; ASIC; 27−34.

Received for review February 21, 2003. Revised manuscript received May 10, 2003. Accepted May 25, 2003.

JF0341767