

The Elusiveness of Coffee Aroma: New Insights from a Non-empirical Approach

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Aroma is central to a pleasurable eating/drinking experience but is one of the most labile components of food. Coffee is an outstanding example. Attempts to avoid or control aroma degradation are often frustrated by ignorance of the microscopic mechanisms that are responsible for it. One of the processes most frequently invoked is radical formation, yet the identity of the radicals and their involvement in aroma degradation are poorly understood at the molecular level. Here a step forward in the fundamental understanding of this complex problem is taken by identifying the most relevant radicals and their products using first-principles calculations. Over 100 radicals originating from key aroma compounds found in coffee and other foods have been studied and classified according to an unambiguous criterion: their thermodynamic stability relative to common radical sources. This classification scheme predicts that most aroma molecules are resistant to both peroxidation and attack from phenolic antioxidants but are unstable with respect to radicals such as $\cdot\text{OH}$. Dimers—generated from radical reactions—were also considered, and the most volatile species, which may further contribute to coffee aroma degradation, were focused on. Those—which are very few indeed—have this potential have been identified.

KEYWORDS: Aroma; flavor; coffee; volatility; furfurylthiol; radical formation; Fenton chemistry

INTRODUCTION

Enticing aromas are undoubtedly a pleasant note in daily life. However, we all know that they are ephemeral: preserving a good aroma and preventing it from deteriorating and fading away are indeed severe problems (1). Currently we are able to say what causes the rich aroma of freshly prepared coffee, but we know too little about why and how it degrades after just 3 h (2). The complexity of this type of problem deters research using conventional methods. The primary challenge is to find a way of reducing it to a handful of subproblems that are more directly approachable. Making progress by studying, in whatever detail or accuracy, only one chemical compound or reaction is hopeless, and studying all possible channels leading to degradation reactions simultaneously is unrealistic.

One of the processes that is most frequently invoked to explain aroma degradation is the radical formation route. Radical-mediated processes undoubtedly play a key role (3–8) because radical sources are ubiquitous and free radicals are byproducts of the metabolism of all living organisms. Once radical species are formed from the key flavor compounds, they may affect the aroma in various ways, for example, by

decreasing the concentration of specific aroma impact molecules, by revealing an intrinsic off-flavor characteristic, and by generating new off-flavor compounds via reactions with a variety of compounds including other aroma radicals and nonvolatiles in the coffee matrix, as well as by initiating a chain of radical reactions. The first step toward shining light into this complex mixture of events is the identification of the radicals themselves and of their possible roles. Experiments encounter an unavoidable difficulty in identifying radical species out of a multitude, because of their short lifetime. Moreover, their detection via electron paramagnetic resonance (EPR) measurements (9, 10) is made difficult by their dependence on the physical and chemical conditions in which experiments are made as well as on spectral resolution and on the spin-trap selected. Therefore, we believe that an approach based on accurate computer simulations rather than on empirical data is a valuable tool for the investigation of the radical formation route.

The aim of this paper is to provide rational, general, and accurate guidelines to identify likely radicals that originate from aroma volatile compounds and also determine the most probable products of subsequent radical–radical reactions that may contribute to the degradation process. We consider a whole set of volatile flavor compounds (24 in total) found in coffee (2, 11) and in a great variety of other food products as well (see,

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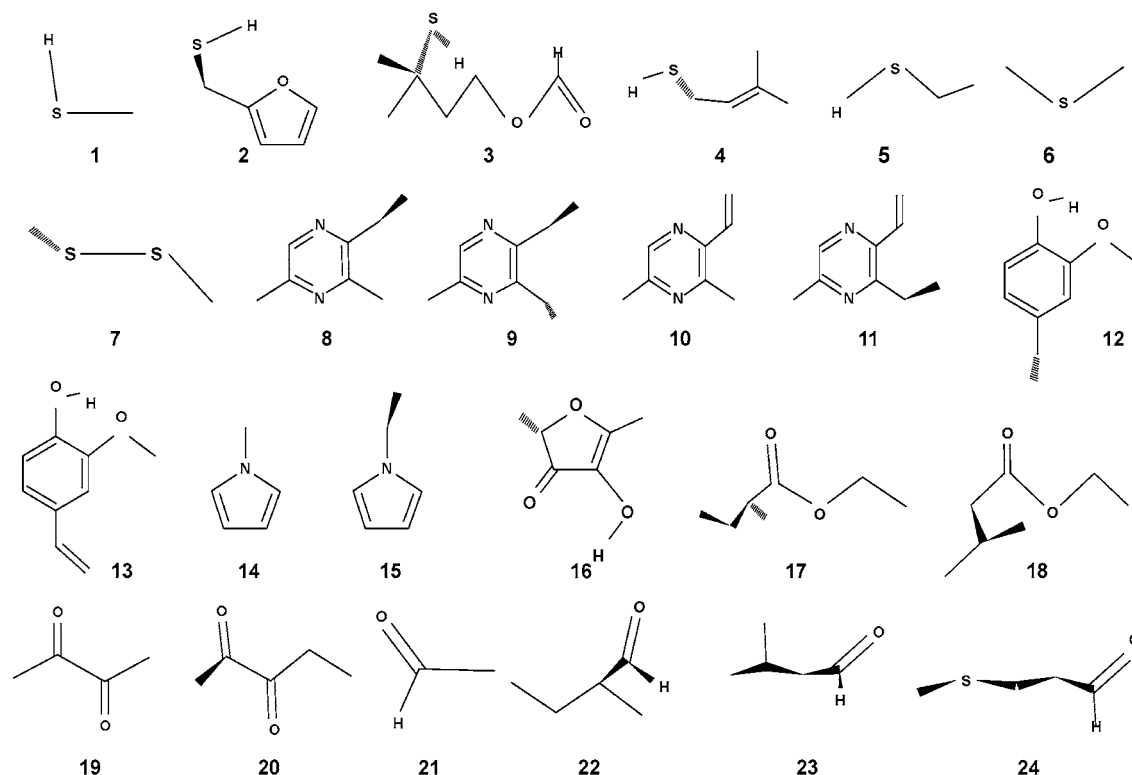


Figure 1. Aroma compounds: (1) methanethiol; (2) 2-furfurylthiol (FFT); (3) 3-mercapto-3-methylbutyl formate; (4) 3-methyl-2-butenethiol; (5) ethanethiol; (6) dimethyl sulfide; (7) dimethyl disulfide; (8) 2-ethyl-3,5-dimethylpyrazine; (9) 2,3-diethyl-5-methylpyrazine; (10) 2-vinyl-3-ethyl-5-methylpyrazine; (11) 2-vinyl-3-ethyl-5-methylpyrazine; (12) 4-ethyl-2-methoxyphenol; (13) 4-vinyl-2-methoxyphenol; (14) *N*-methylpyrrole; (15) *N*-ethylpyrrole; (16) 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol); (17) ethyl 2-methylbutyrate; (18) ethyl 3-methylbutyrate; (19) 2,3-butanedione; (20) 2,3-pentanedione; (21) ethanal; (22) 2-methylbutanal; (23) 3-methylbutanal; (24) 3-(methylthio)propanal (methional).

e.g., 12–16). Radical species can be generated with several mechanisms, such as electron transfer or radical addition, but the most likely to form from a kinetic viewpoint are those created by hydrogen extraction. Therefore, first we examine all 107 distinct radicals that can be formed by removing one hydrogen from the 24 molecules and classify them according to their thermodynamic stability. Next we investigate the dimer products of the reaction between selected radicals in terms of their chemical and thermodynamic stabilities and estimate their volatility with specific reference to coffee. Again, our choice of considering here dimer products rather than other combinations of radicals is dictated by the fact that they have a higher probability of formation and also lower molecular masses. Whereas our classification of radicals is relevant to various forms of coffee, for example, roasted and brewed coffees, prediction of dimer products is expected to be particularly useful when the reactivity of a coffee aroma extract is considered.

METHODS

Our computational scheme is based on density-functional theory (17) with gradient-corrected and spin-polarized exchange and correlation functionals. The specific choice we made for the functional approximations (18, 19) is known to give a good level of accuracy for the energetics of organic molecules. Norm-conserving angular-momentum-dependent pseudopotentials were used to represent the interaction of valence electrons with the atomic cores, and a plane-wave basis set was used for the expansion of the electronic valence wave functions, up to a kinetic energy cutoff of 70 Ry. We refer the reader to ref 20 for a discussion of the method and its previous applications to a variety of other problems. Calculations were performed using the Car-Parrinello Molecular Dynamics (CPMD) code (21).

Within this scheme, we optimized the structure of the radical and dimer species in the gas phase and calculated both their formation

energies and their electronic properties. We have also performed some calculations accounting for the presence of the solvent, using several continuum models (22, 23).

Estimates of the volatility of the dimer species were made using the bond contribution method (24–26), which was originally validated on >400 compounds. It needs only the chemical structure of a molecule as input, and its accuracy is sufficient for our purposes.

RESULTS AND DISCUSSION

We consider a set of 24 volatile compounds that are known to have a significant impact on the aroma of many foods, and notably coffee. A sketch of the molecules is given in **Figure 1**. These represent examples from nine different classes: thiols (1–5), sulfides (6, 7), pyrazines (8–11), 2-methoxyphenols (12, 13), pyrroles (14, 15), furanones (16), esters (17, 18), diketones (19, 20), and aldehydes (21–24).

The structure–activity relationship and the influence on the nature and quality of the aroma they create have been the subject of intensive experimental and theoretical studies (1, 27). We take one step further and investigate what happens when these compounds come into contact with a radical source. Independent of their composition, radicals will form only if their formation free energy does not exceed that of the source. Thus, thermodynamics offers a simple criterion to bring order to this complex set of data. In addition, analysis of the structures in **Figure 1** helps to identify some of the intrinsic kinetic factors that would affect the rate of formation, for example, a high number of equivalent hydrogens (isomeric degeneracy) or the extent of steric hindrance. The kinetic aspects, however, are less important here because we are interested in the response of the systems at sufficiently long times so that thermodynamic equilibrium is attained.

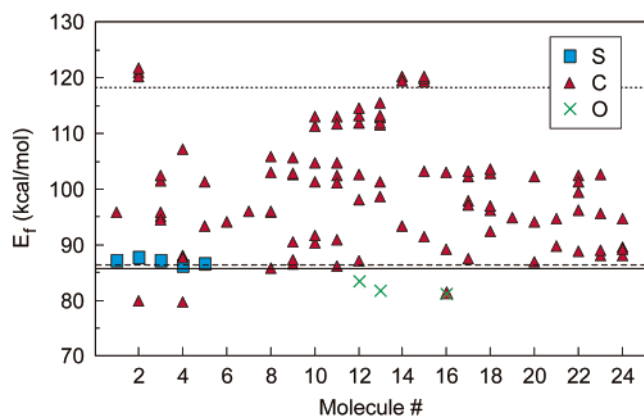


Figure 2. Formation energies (in kilocalories per mole) calculated for all radicals obtained by extracting one hydrogen from the aroma compounds shown in **Figure 1**. (Further details are reported in the Supporting Information.) The reference lines indicate the values calculated for the formation of a hydroxyl radical ($\bullet\text{OH}$) from water (dotted line), a perhydroxyl radical ($\text{HO}_2\bullet$) from hydrogen peroxide (dashed line), and a semiquinone radical ($\text{PhO}\bullet$) from phenol (solid line) (see text).

By considering only the extraction of one H, as many as 107 distinct radicals are generated. We have calculated their formation energies in the gas phase. Using several continuum models for the solvent (22, 23), we have also checked that solvent effects do not alter their final distribution. Moreover, we do not expect entropic effects to change this picture: the entropy differences should not be significant compared with the enthalpy differences in view of the similarity of the molecules.

In **Figure 2** we report the computed values of their formation energies (E_f) (28) and compare them with those of $\bullet\text{OH}$ (119 kcal/mol), $\text{HO}_2\bullet$ (87 kcal/mol), and $\text{PhO}\bullet$ (86 kcal/mol) (29). These radicals are meant to represent all alcoholic, peroxidic, and phenolic radicals that are expected to be present in foods and act as free radical scavengers. From **Figure 2** one can argue that, apart from a few exceptions, all aroma radicals will form when $\bullet\text{OH}$ is the source. However, when the source is either $\text{HO}_2\bullet$ or $\text{PhO}\bullet$, far fewer radicals are predicted to form. In summary, most aroma impact compounds are predicted to be resistant to both peroxidation and attack from phenolic antioxidant molecules but are clearly unstable with respect to radicals such as $\bullet\text{OH}$. This suggests that to preserve the integrity of coffee aromas one should minimize Fenton reaction chemistry.

The few radicals with $119 < E_f < 122$ kcal/mol are unlikely to form under Fenton chemistry conditions: they are a subset of those derived from 2-furfurylthiol (molecule **2** in **Figure 1**) and from both pyrroles (molecules **14** and **15** in **Figure 1**). We notice that they have an interesting feature in common, namely, that they all involve the removal of hydrogen from a five-membered heterocyclic ring. Most radicals in the “intermediate” region ($87 < E_f < 115$ kcal/mol) are carbon-centered and may exhibit several equivalent hydrogens.

The “thermodynamic” classification scheme identifies six radicals as the most probable ones: two on thiols (**2** and **4**), two on 2-methoxyphenols (**12** and **13**), and two on 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**16**). This list includes one of the most widely studied aroma compounds, 2-furfurylthiol (FFT; **2**). It has been reported to be a volatile constituent of many foods and beverages (e.g., meat, coffee, cocoa, malt, popcorn, and wine) (14–16), especially when their production involves thermal treatment. Our calculations demonstrate that the lowest energy FFT radical ($E_f = 80$ kcal/mol) is not centered on sulfur, as commonly assumed (30), but on the carbon adjacent to the aromatic ring. This is favored by 8 kcal/mol, in agreement with delocalization on the aromatic ring. EPR experiments on FFT under Fenton-type reaction conditions detected the presence of C-centered radicals but not that of S-centered ones; this unexpected observation was attributed to spin-trap selectivity (9). Similarly for 3-methyl-2-butenethiol (**4**), we find that the lowest energy radical ($E_f = 80$ kcal/mol) is centered on the allylic carbon and is more stable than the sulfur-centered radical by 6 kcal/mol. The 2-methoxyphenol examples ($E_f = 82$ and 84 kcal/mol for **13** and **12**, respectively) are oxygen-centered and are not surprising because phenolic radicals are expected to be particularly stable. This is also true for the tertiary carbon ($E_f = 82$ kcal/mol) and the vinyl alcohol radicals of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone ($E_f = 81$ kcal/mol). Their degeneracy was less predictable but is consistent with the similar degree of electron delocalization in the conjugated system.

The thermodynamic classification scheme in **Figure 2** also helps us to categorize the most likely processes these radicals may undergo. Those with higher formation energies will have shorter lifetimes and can more easily extract a hydrogen from other molecules, potentially triggering a chain reaction. The ones with the lowest formation energy—which will also result from the above sequence of reactions—are those which will have the longest lifetimes and thus may be involved in coupling reactions with higher probability. Moreover, the more stable the radical,

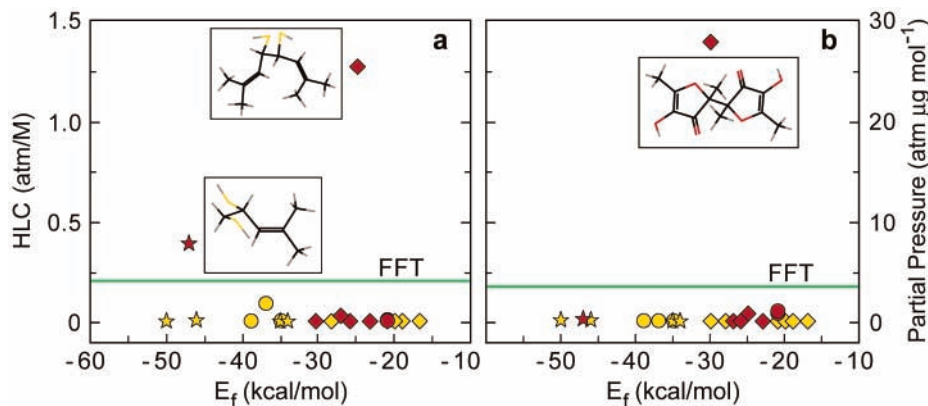


Figure 3. (a) Henry's law constant (HLC, air-to-water partition coefficient) versus formation energy: combinations of stable radicals (diamonds); combinations with methanethiol radical centered on S (circles) or C (stars); chemically stable (red) or unstable (yellow) species. (b) Upper limit of partial pressure estimated in coffee for the dimers in (a) versus formation energy (Further details are reported in the Supporting Information). Concentrations of molecules in coffee brew were taken from ref 17. In both (a) and (b) the green line refers to 2-furfurylthiol (FFT). Insets show the structure of the remarkable cases represented with sticks; red, black, and yellow denote oxygen, carbon, and sulfur, respectively.

the fewer the molecules from which hydrogen extraction is thermodynamically favorable, which renders coupling increasingly probable.

Among the products of reactions between radicals, dimers are expected to form more readily (31). Thousands of dimers are generated from the coupling of the 107 radicals (32), but we focus here on those resulting from the coupling of the more stable, and thus longer living, radicals with each other and also with methanethiol, because of its potential to form volatiles. The most stable dimers are those of interest because—depending on their volatility—they have the potential to introduce new overtones to either the aroma or the food matrix. We identified the most thermodynamically and chemically stable of the selected set by calculating their formation energies (see Supporting Information) and determining their propensity to react on the basis of the electronic structure (32). Only one-third satisfy both of our thermodynamical and chemical stability criteria. As expected, the three dimers that involve the formation of a peroxide bond are energetically unfavorable compared with the reactants. From the analysis of the frontier orbitals, approximately half of the dimers appeared to be susceptible to either oxidation or reduction processes.

To examine the potential impact of these novel compounds on the aroma more directly, we have estimated their volatility (which is proportional to the Henry's law constant) by applying the bond contribution method (24–26) to our chemical structures. When these values are compared with that of a fairly volatile molecule such as FFT (Figure 3a), only two dimers appear to be relevant, both of which fall in the “stable” set. They are formed from combinations of the C-centered radical of 3-methyl-2-butenethiol (4) either with itself ($E_f = -25$ kcal/mol) or with the C-centered radical of methanethiol (1) ($E_f = -47$ kcal/mol). To gain a more direct estimate of the potential impact for a specific system, one can convert volatilities into maximum partial pressures (partial pressure_{max} = volatility × [radical]_L, where [radical]_L is the concentration of whichever radical component of the dimer has the lowest concentration in coffee in its molecular form). This is shown in Figure 3b for coffee. This time another “stable” molecule emerges: the one formed by dimerization of the tertiary carbon radical of 4-hydroxy-2,5-dimethyl-3(2H)-furanone ($E_f = -30$ kcal/mol), which is present in its molecular form in large concentrations in coffee (11).

Our results to date and the procedure illustrated here suggest a new program of research in food science in which calculations of the type presented here would complement the experimental effort. By laying a path through the complex scenario of food flavor, our findings lead to new experiments aimed at detecting the compounds we have identified and characterized. The relevance of these unforeseen compounds may manifest itself not only in the loss of food's fresh aroma but also in the deterioration of its taste. In addition, by providing a rational and quantitative approach at the atomic level, our calculations should ultimately be useful to conventional modeling of real food systems. The next challenge will be to explore the influence on radical formation of environmental factors and competing processes, for example, hydrolysis.

Supporting Information Available: Tables listing the composition and formation energies for the radicals discussed in the text and the dimers in Figure 3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (29) The calculated E_t values (119, 87, and 86 kcal/mol) correlate well with the experimental bond dissociation energies measured at room temperature for HO–H (119 kcal/mol), HO₂–H (88 kcal/mol), and PhO–H (86.5 kcal/mol) (*CRC Handbook of Chemistry and Physics*, 75th ed.; Lide, D. R., Ed.; CRC: Boca Raton, FL, 1995).
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- (32) Calculations on a more extended set of dimers generated by the 107 radicals considered in this paper will be the subject of a subsequent publication.

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