

# Volatile Compounds in Archaeological Plant Remains and the Maillard Reaction During Decay of Organic Matter

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Archaeological plant remains from excavations at Qasr Ibrîm, Egypt, preserve volatile decay products trapped within internal networks of structural and storage macromolecules. These volatile components can be linked to specific degradative reactions occurring during the long-term burial of organic matter. Abundant alkyl pyrazines are characteristic by-products of the Maillard (or browning) reaction of proteins and carbohydrates and provide evidence for the reaction occurring in buried organic matter.

Qasr Ibrîm is the site of a major archaeological settlement located on the banks of the Nile in Egyptian Nubia. Excavations have yielded vast quantities of paleobotanical remains, including cereals, legumes, and oil seeds, that display exceptional morphological preservation. These plant remains provide data on the various phases of occupation of the settlement (1) and are also proving to be useful model systems for studying aspects of biomolecule degradation. Examination of the structure of propagule outer walls (seed coat and fruit wall) by scanning electron microscopy showed no visible signs of fungal or bacterial attack (2). The only discernible sign of deterioration was that the ancient specimens had shrunk relative to modern specimens (Fig. 1). Biomolecular analyses have shown that compositional changes occurred during the period of burial. Evidence of hydrolysis is seen in the conversion of triacylglycerols to free fatty acids, whereas a reduction in the abundance of unsaturated fatty acids indicates free-radical oxidative damage (2, 3). Intact purine and pyrimidine bases (4) and nucleosides (5) have been identified in the propagules, although the DNA strands are highly fragmented (3).

When we macerated propagules of various species (eight species, including three varieties of sorghum, ranging up to 1500 years in age), we noted a pungent odor that was not produced by modern specimens. We had not noticed such an odor in our studies of other archaeological and fossil plant remains, and its nature suggested that heteroatomic organic compounds were

abundant in the samples. We collected the volatile components on Tenax cartridges for analysis by desorption headspace gas chromatography-mass spectrometry (GC-MS) (6).

Two examples of the taxa we studied—*Hordeum* sp. (barley, monocotyledon) and *Ra-*

*phanus sativum* (radish, dicotyledon)—emitted a wide variety of volatile compounds, including various alkyl pyrazines [methylpyrazine, 2,5- and 2,6-dimethylpyrazine, ethylmethylpyrazine (two isomers), trimethylpyrazine, ethyldimethylpyrazine (three isomers), 2,5-diethylpyrazine, 2,6-diethyl-3-methylpyrazine, and other C<sub>4</sub> and C<sub>5</sub> alkyl pyrazines], alkyl polysulfides (CH<sub>3</sub>S<sub>2</sub>CH<sub>3</sub>, CH<sub>3</sub>S<sub>3</sub>CH<sub>3</sub>, and CH<sub>3</sub>S<sub>4</sub>CH<sub>3</sub>), and a series of short chain (C<sub>6</sub> to C<sub>10</sub>) oxygenated compounds (including alkan-2-ones and *n*-alkanals) (Fig. 2). These volatile species were not detected in freshly ground modern propagules of either taxon or in analytical blanks and must be physically occluded within macromolecular networks; if they were free to diffuse, they would have been lost during burial. Adsorption through van der Waals interactions or hydrogen bonding may play a part in retaining the low-molecular weight constituents; however, these interactions must be relatively weak because the volatile compounds were released immediately on crushing the propagules. The presence of the dimethylsulfides



**Fig. 1.** Light microscope photographs of cross sections cut through the ancient propagules from Qasr Ibrîm, *Hordeum* sp. (A) and *Raphanus sativum* (B), compared with those of their modern counterparts (C and D). The shrinkage of the ancient specimens, most evident in (A), is the result of desiccation. The darkening of the internal storage tissues provides visible evidence of biomolecular decay through the Maillard or browning reaction. Evidence for this reaction occurring within the propagules comes from the characteristic volatile compounds released upon crushing and shown in Fig. 2.

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accounts for the pungent odor. The high abundance of proteins (7) in the radish seeds correlates with the appreciably higher abundance of dimethylpolysulfides associated with this taxon, compared with those for ancient barley. The volatile oxygenated compounds (alkanals and alkan-2-ones) derive from autoxidation of unsaturated, particularly polyunsaturated, fatty acids (8).

Some 80 years ago, Maillard investigated the origin of dark brown products, the so-called melanoidins, formed during reactions of sugars and amino acids (9–12). This reaction is now known as the Maillard or browning reaction. These high-molecular weight heteropolymeric products have since been shown to have commercial, nutritional, and toxicological significance (13). Important by-products of the reaction are complex mixtures of low-molecular weight volatile organic compounds, best known for contributing favorable odors to cooked foods. Alkyl pyrazines formed through the Maillard reaction of proteins and carbohydrates are major components of these volatiles (13, 14). The major route to the alkyl pyrazines is by condensation of dicarbonyl compounds (derived from hexose sugars by way of the Amadori rearrangement) with amino acids through Strecker degradation (15). Alkyl pyrazines are uncommon in nature [other than as components of some pheromones (for example, 16)], so their presence indicates that the Maillard reaction produced these chemical transforma-

tions in the ancient propagules. The Maillard reaction is also responsible for the formation of the linear alkyl polysulfides by way of Strecker degradation of sulfur-containing amino acids, most notably cysteine (17). Alkyl furans of the type seen in the ancient barley volatiles arise in the Maillard reaction through dehydration and fragmentation of simple sugars (14).

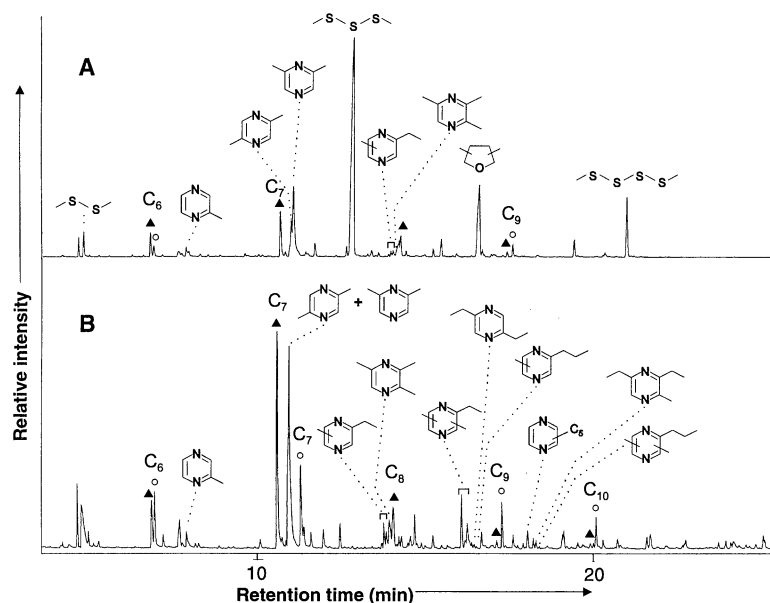
These observations have implications for understanding the transformation of organic matter over time. Several models of organic matter transformation and preservation have been proposed (18–20). In the “neogenesis” model, sedimentary organic matter (for example, humic substances and kerogen) is produced primarily by the random recombination of small labile organic molecules, such as simple sugars and amino acids, to produce refractory geopolymers (18). Although the Maillard reaction has been widely implicated in the formation of humic materials, its role has remained speculative (21, 22) because of the difficulty of characterizing the heterogeneous geopolymers (which may comprise polymeric melanoidins). Our findings confirm that this reaction occurs during the decay of buried plant materials. The volatile alkyl pyrazines would normally diffuse away from their site of initial formation during decay, once the structural integrity of the plant tissues had been lost. They are, however, retained in the propagules from Qasr Ibrim because the arid burial conditions have slowed disaggre-

gation, thereby ensuring preservation by encapsulation within internal networks of structural and storage macromolecules. Visible evidence for the classical (and complementary) heteropolymeric browning products of the Maillard reaction (melanoidins) is apparent from the coloration of the storage tissues of the ancient propagules seen in the light micrographs (Fig. 1).

## REFERENCES AND NOTES

1. P. Rowley-Conwy, in *New Light on Early Farming*, J. Renfrew, Ed. (Edinburgh Univ. Press, Edinburgh, 1991), pp. 191–212.
2. P. F. van Bergen, H. A. Bland, M. C. Horton, R. P. Evershed, *Geochim. Cosmochim. Acta* **61**, 1919 (1997).
3. K. O'Donoghue, A. Clapham, R. P. Evershed, T. A. Brown, *Proc. R. Soc. London* **263**, 541 (1996).
4. K. O'Donoghue, T. A. Brown, J. F. Carter, R. P. Evershed, *Rapid Commun. Mass Spectrom.* **8**, 503 (1994).
5. ———, *ibid.* **10**, 495 (1996).
6. The volatiles were released by crushing whole propagules in an all-glass headspace extraction vessel cooled with dry ice. After warming to 35°C, the components were collected on a Tenax cartridge by flushing with a stream of dry charcoal-filtered nitrogen. The volatiles desorbed from the cartridge (250°C) were cryogenically focused at the head of the GC column. Analyses were performed with a Carlo Erba 4130 gas chromatograph coupled to a Finnigan 4500 mass spectrometer. Compounds were separated with a CP Sil-19 capillary column (50 m, inside diameter 0.32 mm, film thickness 0.2 µm). The gas chromatograph oven temperature was initially held at 35°C for 4 min, then raised at a rate of 4°C per minute<sup>-1</sup> to 300°C and held for 15 min. The mass spectrometer was operated in the full-scan mode [70 eV, mass/charge ratio (*m/z*) range 35 to 550 at one scan per second]. Components were identified from comparison with library and literature spectra and the known fragmentations of organic compounds.
7. M. Laroche, L. Aspart, M. Delseny, P. Penon, *Plant Physiol.* **74**, 487 (1984).
8. M. G. Simic, S. V. Jovanovic, E. Niki, *Am. Chem. Soc. Symp. Ser.* **500**, 14 (1992).
9. L. C. Maillard, *Compt. Rend.* **155**, 1554 (1912).
10. ———, *Compt. Rend. Soc. Biol.* **72**, 599 (1913).
11. ———, *Ann. Chim. (Paris)* **11** (no. 5), 258 (1916).
12. ———, *ibid.* (no. 7), 113 (1917).
13. J. Mauron, *Prog. Food Nutr. Sci.* **5** (nos. 1–6), 5 (1981).
14. D. S. Mottram, *Am. Chem. Soc. Symp. Ser.* **543**, 104 (1994).
15. H. Weenen *et al.*, *ibid.*, p. 142.
16. R. P. Evershed, E. D. Morgan, M. C. Gammaerts, *Naturwissenschaften* **67**, 374 (1981).
17. G. Vemin and C. Parkanyi, in *Chemistry of Heterocyclic Compounds in Flavours and Aromas*, G. Vemin, Ed. (Ellis Harwood, Chichester, UK, 1982), pp. 151–207.
18. B. P. Tissot and D. H. Welte, *Petroleum Formation and Occurrence* (Springer-Verlag, Berlin, ed. 2, 1984).
19. E. W. Tegelaar, J. W. de Leeuw, S. Derenne, C. Largeau, *Geochim. Cosmochim. Acta* **53**, 3103 (1989).
20. R. G. Keil, D. B. Montjucon, F. G. Prah, J. I. Hedges, *Nature* **370**, 549 (1994).
21. R. Ikan, Y. Rubinsztain, A. Nissenbaum, I. R. Kaplan, in *The Maillard Reaction: Consequences for the Chemical and Life Sciences*, R. Ikan, Ed. (Wiley, Chichester, UK, 1996), pp. 1–25.
22. M. J. Collins, P. Westbroek, G. Muyzer, J. W. de Leeuw, *Geochim. Cosmochim. Acta* **56**, 1539 (1992).
23. We thank J. R. Maxwell and D. E. G. Briggs for their comments, the Egyptian Exploration Society for ancient materials, and the Natural Environment Research Council for MS facilities (grants GR3/2952, GR3/3758, FG6/36/01), a research grant (GR3/9578, R.P.E.), and a studentship (H.A.B.).

25 June 1997; accepted 2 September 1997



**Fig. 2.** Partial desorption headspace GC-MS total ion chromatograms for the volatile compounds trapped in the ancient propagules (**A**) radish (*Raphanus sativum*) and (**B**) barley (*Hordeum* sp.). The structures of the alkyl pyrazines and alkyl polysulfides, characteristic of the Maillard reaction and referred to in the text, are shown adjacent to the peaks on the chromatogram. The range of aliphatic carbon compounds (annotated on the chromatograms with their chain length) refers to alkan-2-ones (▲) and *n*-alkanals (○) formed through lipid oxidation.