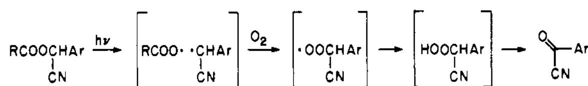
Photolysis of rigidly deoxygenated alcohol solutions does not produce the benzoyl cyanide **12**, establishing that the oxygen source is external and negating our previously proposed mechanism (Ruzo et al., 1977). However, **12** and its degradation products (**13**) are observed even in the presence of trace amounts of oxygen. Thus, a likely pathway for formation of **12** involves a hydroperoxide as



shown in the scheme above. The phenoxybenzoyl cyanide can then react with solvent to give the corresponding acid (**13**, R = H) or ester (**13**, R = Me) (Ruzo et al., 1977; Holmstead et al., 1978b).

The products resulting presumably from photonucleophilic reaction with solvent, i.e., **8** and cyanohydrin **9**, form equally in the presence or absence of oxygen. The cyanohydrin could be observed directly in this study upon derivatization with acetic anhydride: CI-MS m/z (rel intensity) 268 (M + 1, 4), 241 (M – CN, 42), 208 (M – AcO, 100). **9** decomposes further to the aldehyde (**14**).

Micellar solutions yield simple product mixtures containing the dihalovinyl acid (**8**, R = H) and 3-phenoxybenzaldehyde and acid (**13**, R = H and **14**). It appears that in close proximity to an abundant hydrogen source the carboxylate radical abstracts hydrogen readily to give the acid **8** (R = H), while the corresponding cyanobenzyl radical is longer lived and can undergo secondary reaction with oxygen to give the sequence outlined in the scheme. The 3-phenoxybenzaldehyde and part of the acid **8** (R = H) obtained must arise from nucleophilic cleavage of the ester and subsequent decomposition of the cyanohydrin (Ruzo et al., 1977).

Oxidation products are also formed by reactions in the vinyl side chain. These caronic acid derivatives (**4–6**) do not arise from dioxetanes formed on singlet oxygen addition since cypermethrin is unreactive to dye-generated $^1O_2$. They are more likely to form by reaction with ozone generated in solution (Ruzo and Casida, 1982).

The present results illustrate the lability of the dihalovinyl group of pyrethroids under oxidative conditions and clarify the reasons for depletion of the acid moiety and for the formation of **12**, a product common to all α-cyano-3-phenoxybenzyl pyrethroid esters studied (Ruzo, 1982a).

ACKNOWLEDGMENT

I thank John Casida and William Draper of this laboratory for helpful discussions regarding this work.

Registry No. 1 (Y = C, X = Cl), 52315-07-8; 1 (Y = N, X = Cl), 68523-18-2; 11 (R = Me), 85629-14-7.

LITERATURE CITED

Graham-Bryce, I. J. In "Pyrethroid Insecticides: Chemistry and Action, Tables Rondes"; Roussel Uclaf: Paris, 1980; Vol. 37, p 57.

Holmstead, R. L.; Casida, J. E.; Ruzo, L. O.; Fullmer, D. G. *J. Agric. Food Chem.* **1978a**, *26*, 590.

Holmstead, R. L.; Fullmer, D. G.; Ruzo, L. O. *J. Agric. Food Chem.* **1978b**, *26*, 954.

Mikami, N.; Miyamoto, J. *Proc. IUPAC, 5th* **1982**, in press.

Miyamoto, J. *Pure Appl. Chem.* **1981**, *53*, 1967.

Ruzo, L. O. In "Progress in Pesticide Biochemistry"; Hutson, D. H.; Roberts, T. R., Eds.; Wiley: New York, 1982a; Vol. 2, p 1.

Ruzo, L. O. *Proc. IUPAC, 5th* **1982b**, in press.

Ruzo, L. O. *Proc. IUPAC, 5th* **1982c**, in press.

Ruzo L. O.; Casida, J. E. *J. Agric. Food Chem.* **1982**, *30*, 963.

Ruzo, L. O.; Holmstead, R. L.; Casida, J. E. *J. Agric. Food. Chem.* **1977**, *25*, 1385.

Luis O. Ruzo

Pesticide Chemistry and Toxicology Laboratory
Department of Entomological Sciences
University of California
Berkeley, California 94720

Received for review November 8, 1982. Revised manuscript received February 17, 1983. Accepted March 10, 1983. This work was funded in part by a grant from the National Institutes of Environmental Health Sciences (PO1 ES00049).

Inhibition of Sugar–Amine Browning by Aspartic and Glutamic Acids

The browning of lysine–glucose and lysine–fructose model systems (pH 8.0, 60 °C, 58 h) was decreased by adding L-aspartic acid or L-glutamic acid. Specially prepared potato chips darkened less when they were dipped in aspartic or glutamic acid solutions before frying.

Since Maillard (1912) first observed the darkening accompanying the reaction of sugars with amino acids, numerous publications appeared discussing the so-called nonenzymatic browning of foods (Hodge, 1953; Ellis, 1959; Shallenberger and Birch, 1975). It is now known that, besides the carbonyl–amine reaction, several other nonenzymatic reactions can lead to food browning and that some of the browning reactions may also affect the flavor and the nutritional value of foods.

In this communication, we report on the observation that L-aspartic acid and L-glutamic acid may significantly diminish the browning resulting from the interaction of an amino acid, lysine, strongly involved in food darkening, with two common food sugars, glucose and fructose. This observation was made during a larger study in which amino acids, single or in pairs, were allowed to react with glucose and fructose.

EXPERIMENTAL SECTION

The following eight groups of reaction systems were prepared by dissolving the appropriate quantities of reagents in 0.2 M phosphate buffer, pH 8.0: (a) 0.4 M D-glucose, 0.04 M L-lysine, and L-aspartic acid at seven concentrations in the range 0.00–0.04 M; (b) 0.4 M D-

0021-8561/83/1431-1115$01.50/0 © 1983 American Chemical Society

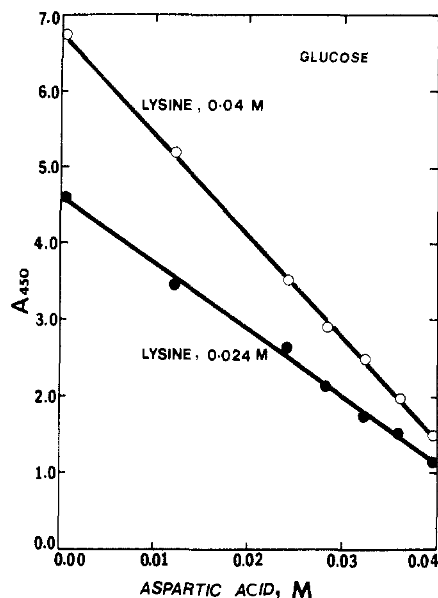


Figure 1. Effect of aspartic acid on the color (A_{450}) of a glucose (0.4 M)–lysine (0.04 M, 0.024 M)–phosphate buffer (pH 8.0) system after 58 h of incubation at 60 °C.

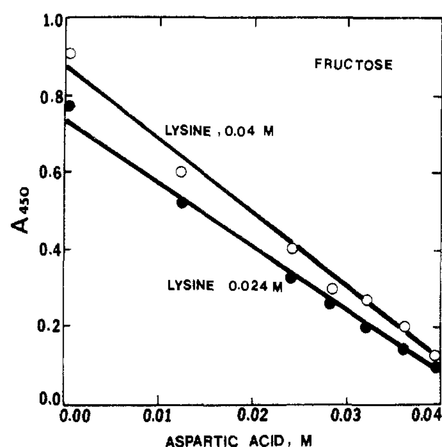


Figure 2. Effect of aspartic acid on the color (A_{450}) of a fructose (0.4 M)–lysine (0.04 M, 0.024 M)–phosphate buffer (pH 8.0) system after 58 h of incubation at 60 °C.

glucose, 0.024 M L-lysine, and L-aspartic acid spanning the same concentration range as in (a); (c) 0.4 M D-fructose, 0.04 M L-lysine, and again L-aspartic acid as in (a); (d) 0.4 M D-fructose, 0.024 M L-lysine, and once again L-aspartic acid as in (a). The remaining four groups of model systems were similar in composition to the first ones except that L-glutamic acid was added instead of L-aspartic acid to the sugar–amine systems. Eight-milliliter aliquots of each system were transferred into 10-mL ampules which were then sealed by flame and placed in a water bath thermostated at 60 °C. After 58 h of incubation the ampules were opened and the absorbance of their content was measured at 450 nm in a Beckman DU spectrophotometer after appropriate dilution in order to bring the A_{450} readings within the 0.1–1.0 range. Absorbance at 450 nm is used as a measure of browning.

In a more applied experiment, potatoes (cv. Russet Burbank) were cut to 2 mm thick slices, which were then freeze-dried, soaked in phosphate buffer, pH 8.0, containing 0.04 M L-asparatic acid or 0.04 M L-glutamic acid, deep-fried at 210 °C for 7 min, coarsely ground, and measured for reflected color both in a Hunter Color Difference meter and in a Spectronic 505, equipped with a reflectance attachment.

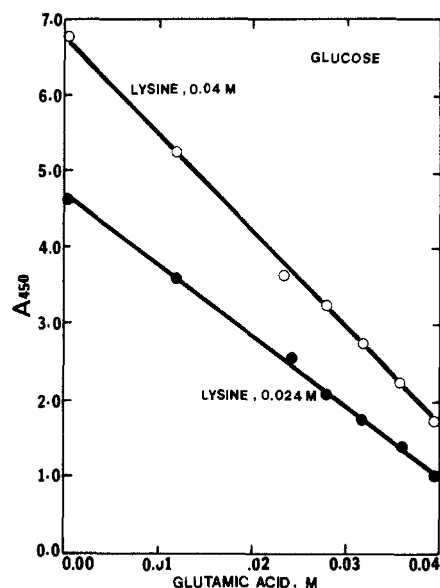


Figure 3. Effect of glutamic acid on the color (A_{450}) of a glucose (0.4 M)–lysine (004 M, 0.024 M)–phosphate buffer (pH 8.0) system after 58 h of incubation at 60 °C.

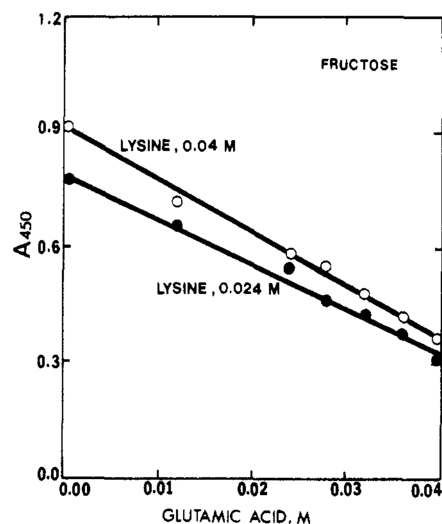


Figure 4. Effect of glutamic acid on the color (A_{450}) of a fructose (0.04 M)–lysine (0.04 M, 0.024 M)–phosphate buffer (pH 8.0) system after 58 h of incubation at 60 °C.

RESULTS AND DISCUSSION

The effect of various concentrations of aspartic acid or glutamic acid on the color (A_{450}) of glucose–lysine and fructose–lysine reaction systems at pH 8.0, after 58 h of incubation at 60 °C, is shown in Figures 1–4. In all systems the browning was greatly reduced as the concentration of the dicarboxylic acid increased. The decrease was slightly more precipitous in the systems containing the higher of the two lysine concentrations. It may also be noticed that the reaction of glucose with lysine resulted in more intense browning than that of fructose with lysine; this is consistent with previous observations (Lewis and Lea, 1950). The Hunter L values (Table I), which are a measure of the lightness or darkness of a surface, indicate that potato chips treated with aspartic acid or glutamic acid are lighter in color than untreated chips. The results are corroborated by the Spectronic 505 reflectance spectra which show that the treated chips reflect 12–15% more light than the control chips in the 420–620-nm wavelength range. Visual observation agreed with the instrument measurements.

Table I. Hunter L Values of Control and Treated Potato Chips[a]

replicate	control	dipped in aspartic acid	dipped in glutamic acid
1	39.0[b]	47.6	44.2
2	38.3	44.6	44.7
3	38.2	45.3	43.0
4	39.0	47.0	43.0
5	37.9	46.8	43.7
6	36.7	46.6	43.9
7	39.5	47.2	43.6
mean	38.4 ± 0.57[c]	46.4 ± 1.00	43.7 ± 0.57

[a] The treatment consisted in freeze-drying potato slices and dipping them in 0.04 M aspartic or glutamic acid before frying. [b] Each L value is the average of three readings obtained by rotating the sample at 120° angles. [c] The differences between control and treated samples are significant at the 99% probability level. The difference between treated samples is not significant.

In this demonstration, the potato slices were freeze-dried in order to facilitate the absorption of the amino acid solution. In commercial practice, slight drying of the slice surfaces might be sufficient to accomplish the desired solution absorption.

Registry No. L-Aspartic acid, 56-84-8; L-glutamic acid, 56-86-0; L-lysine, 56-87-1; D-glucose, 50-99-7; D-fructose, 57-48-7.

LITERATURE CITED

Ellis, G. P. *Adv. Carbohydr. Chem.* **1959**, *14*, 63–133.
Hodge, J. E. *J. Agric. Food Chem.* **1953**, *1*, 928–943.
Lewis, W. M.; Lea, C. H. *Biochim. Biophys. Acta* **1950**, *4*, 532–534.
Maillard, L. C. *C. R. Hebd. Seances Acad. Sci.* **1912**, *154*, 66–68.
Shallenberger, R. S.; Birch, G. G. "Sugar Chemistry"; Avi Publishing Co.: Westport, CT, 1975; pp 169–193.

Karim Nafisi[1]
Pericles Markakis*

Department of Food Science and Human Nutrition
Michigan State University
East Lansing, Michigan 48824
[1]Present address: Northville Labs
Northville, MI 48167

Received for review November 29, 1982. Accepted March 28, 1983. Michigan Agricultural Experiment Station Journal Article No. 10657.

Methylation of Deoxyribonucleic Acid in the Rat by the Mushroom Poison Gyromitrin

Gyromitrin (acetaldehyde *N*-methyl-*N*-formylhydrazone) is the most preponderant of a group of hydrazone compounds found in the false morel mushroom *Gyromitra esculenta*. It decomposes to products considered to be metabolized to methylating agents in vivo. [*N-methyl*-^{3}H]Gyromitrin was synthesized from formylhydrazine by reaction with acetaldehyde in absolute alcohol and methylation of the resulting hydrazone with tritiated methyl iodide in tetrahydrofuran. [^{3}H]Gyromitrin was administered orally to young adult male rats. After 6 h, DNA was isolated from liver and lung. Although all DNA samples were radiolabeled, analysis by HPLC of the purines liberated from the DNA upon acid hydrolysis revealed that only liver DNA contained 7-methylguanine as a result of DNA methylation. Most radioactivity coeluted with the natural purines and was the result of biosynthetic incorporation of radiolabeled breakdown products of gyromitrin. Expressed as DNA damage per dose in the units of a Covalent Binding Index, CBI, gyromitrin exhibited an average value of 15 for liver DNA, while the respective value for lung (CBI < 5) was below our limit of detection. Upon comparison with the unavoidable intake of the methylating carcinogen dimethylnitrosamine (CBI = 6000), it is concluded that the false morel mushroom should only be consumed occasionally.

The false morel mushroom *Gyromitra esculenta* is poisonous when eaten fresh. Only after drying or thorough cooking is it considered edible and even then sometimes gives rise to intoxications (Franke et al., 1967). Typical symptoms are nausea, gastroenteritis, vomiting, and icterus (Franke et al., 1967; Guisti and Carnevale, 1974). Chemical analysis of the mushroom revealed that the principal toxic component was gyromitrin (List and Luft, 1967, 1969), subsequently found to be the most preponderant of a series of hydrazone compounds (Pyysalo and Niskanen, 1977). Since these compounds are highly volatile, it is not surprising that drying or cooking the mushrooms significantly reduces the toxicity. Gyromitrin is hydrolyzed in vivo to methylhydrazine (von Wright et al., 1978), a compound which was shown to be carcinogenic in the hamster (Toth and Shimizu, 1973) and mouse (Toth, 1972) after oral administration. Methylhydrazine methylates mouse liver DNA to a small extent (Hawks and Magee, 1974). Another line of research links the carcinogenic effect of gyromitrin to the formation of a nitrosamide (Braun et al., 1980; see Figure 1), a family of compounds known to be carcinogenic through their spontaneous conversion into alkylating agents.

The above considerations strongly suggested a possible genotoxic risk from gyromitrin via either methylhydrazine or nitrosamide formation and subsequent DNA methylation. Indeed, an increased lung tumor rate in Swiss mice was found after oral application of 100 mg of gyromitrin/kg of body weight weekly during their lifetime (Toth et al., 1981).

The aim of our work was to determine the DNA-methylating potency of gyromitrin in vivo and to compare

0021-8561/83/1431-1117$01.50/0 © 1983 American Chemical Society