Modification of Maillard Browning in a Microwaved Glucose/Glycine Model System by Water-Soluble Natural Antioxidants and Foods Containing Them

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ABSTRACT: Inhibition of pyrazine formation by natural antioxidants and the foods containing them was measured in a microwaved glucose/glycine model system. Inhibition of lipid oxidation by the same materials was assayed in both bulk and emulsion systems. Pyrazines were determined by solid-phase micro extraction followed by GC. Lipid oxidation volatiles were assayed by polyamide fluorescence produced by either a bulk oil display or a hematin- or 2,2′-azobis-(2-amidino-propane) dihydrochloride-accelerated lecithin or fish oil emulsion. It was shown that (i) the inhibition of pyrazine formation depends on high concentrations of water-soluble antioxidants; (ii) such antioxidants occur naturally in some foods and are usually polyphenols; (iii) during pyrazine inhibition, oxidized polyphenols show enhanced nonfluorescing browning similar to enzymic browning products; (iv) monophenols, which structurally cannot form quinone polymers on oxidation, inhibit pyrazines with less browning; (v) during the final pyrazine-forming phase of the Maillard reaction, polyphenolics and reducing agents such as glutathione and ascorbic acid are partially consumed with some nutritional loss; (vi) fruit powders of grape seed, grape skin, and red wine are highly pyrazine-inhibitory, steeped blueberry strongly so, but plum purees are moderately pro-pyrazine, and freeze-dried vegetables strongly pro-pyrazine; and (vii) black and green tea infusions are highly inhibitory, whereas spices have mixed effects.

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KEY WORDS: Front-face solid sample polyamide fluorescence, Maillard reaction, oxidation inhibition, pyrazine inhibition, solid-phase micro extraction, water soluble antioxidants.

Pyrazines, which are responsible for the nutty flavor from Maillard sugar–amine browning reactions, may be produced by what appears to be a free radical phase of the reaction (1–3), although this has been challenged by some studies (4–6). Their production can be inhibited by high concentrations of water-soluble antioxidants (7–14). When present with creatinine in cooked meats, however, pyrazines produce mutagens of the imidazoquinoline type (8,10,15–18). The extent of the health risks of the latter is still under study (3,16). Although there have been several studies of pyrazine inhibition by antioxidants, none seems to have been conducted under microwave conditions, although Ji and Bernhard (19) showed that the pyrazines produced by microwaving, and presumably the taste and flavor as well, will be quite different from those compounds, tastes, and flavors produced by heat alone. The aims of this study were (i) to assess the antioxidant effectiveness of several natural antioxidants contained in common foods, in aqueous media under high-temperature conditions similar to those favoring Maillard browning [lecithin emulsions initiated by hematin (iron-releasing) or 2,2′-azobis-(2-amidino-propane) dihydrochloride (AAPH; non-iron-releasing)], by monitoring with front-face polyamide fluorescence (2,20) and (ii) to assess their effectiveness in inhibiting pyrazine formation in a microwaved model system of small enough volume to permit rapid testing of antioxidants and food components at the low concentrations often found in natural materials. Both an open and a sealed system were used to generate both moderate and high temperatures. Parallel to the studies on intact foods and spices of Kikugawa and Kato (18), Milić *et al.* (21), and Vitaglione *et al*. (22), both antioxidant and anti-pyrazine effectiveness of intact foods or extracts containing these compounds was also studied (23). Pursuant to the method of Ibanez and Bernhard (24), solid-phase microextraction (SPME) and GC were used to assess pyrazines. This method is admirably adapted to small sample volume, rapid sampling, and avoidance of the problems of disposal of extraction solvent. The ultimate purpose of this research was to select the best candidates for sauces and marinades, to be used in cooking meat, to decrease potential carcinogen formation. An adjunct purpose was to demonstrate the extent of loss of polyphenolic antioxidants due to Maillard browning. In addition, means were sought to reduce the great enhancement of the normal Maillard brown color due to attendant oxidation of polyphenolics during the Maillard reaction.

MATERIALS AND METHODS

SPME and GC. SPME Fiber Holder was from Supelco (No. 57330-U; Supelco, Bellefonte, PA). SPME fiber was a 1-cm DVB/Carboxen/PDMS Stableflex Fiber from Supelco (No. 57328-U; Supelco, Bellefonte, PA). The GC instrument was a Hewlett-Packard No. 5890 Series II with FID. The column was

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SUPELCOWAX No. 10 (0.32 mm i.d., 60 m length; Supelco). Helium carrier flow was 20 cm/s at 30°C.

UV/vis and fluorescence spectrophotometry. A Gilford Response II UV/VIS spectrophotometer was used. For fluorescence, both an SLM Aminco (Model SPF4800) and a SPEX Fluorolog-2 were used, both in front-face and transmission modes.

Microwave. A Sharp Carousel B microwave oven, 600 W, with rotating carousel, was used at high power. Intensity of exposure was controlled by timing and positioning of sample and control flasks.

Antioxidant materials. Compounds tested for antioxidant effectiveness and pyrazine inhibition were commercial reagent grade and used without further purification. Effective pyrazine inhibition is only shown by potent water-soluble antioxidants. Two trihydric polyphenols, gallic acid and propyl gallate, and three dihydric polyphenols, chlorogenic, caffeic and tannic acids, were tested. The monohydric water-soluble analog of tocopherol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich Chemical Co., Milwaukee, WI) was tested extensively. Two potent nonphenolic water-soluble antioxidants, the reductone ascorbic acid and the sulfhydryl-containing glutathione (25), were also tested.

Food and beverage materials. Food and beverage samples tested were in the form of fruit purees, juices, and freeze-dried fruit; fruit beverage; and vegetable powders. Plum Juicy was a dried plum powder with 3% calcium stearate procured from the California Dried Plum Board (Sacramento, CA). Dried plum puree was procured from Midway Farms (Fresno, Ca). Freeze-dried Hawaiian taro was procured from Wayne Muller of the U.S. Army Natick Soldier Center (Natick, MA). Kale and broccoli were procured from local markets and freeze-dried. Cloves were ground Durkee Whole Cloves (ACH Food Companies, Memphis, TN) procured from local markets, and oregano was procured from Bread and Circus, Inc. (Needham, MA). Herbalox Rosemary Seasoning, type W, and rosmarinic acid were procured from Kalsec, Inc. (Kalamazoo, Mi). Salada black tea (Salada, Inc., Citrus Plant City, FL) and Trader Joe's Green Tea (Trader Joe's Inc., Needham, MA) were infused from tea bags. Wild blueberry powder tested initially was Blue Phenolics (Biosan Laboratories, Inc., Ferndale, MI). Grape seed and grape skin extract powder and red wine concentrate powder were procured from Polyphenolics, Inc. (Fairport, NY). Grape seed extract powder Activin GSE 2000-85, obtained from San Joaquin Valley Concentrates (Fresno, CA), was also tested as confirmation of the results with the Biosan product. Older cranberry extract powder was 1 1/2 yr from date of canning and was procured from Degas Farms (Pembroke, MA). Fresh cranberry extract powder was from Ocean Spray (Lakeville-Middleboro, MA).

Antioxidant effectiveness of chemical compounds. Antioxidant effectiveness was assayed in both bulk (cobalt-activated stripped corn oil) and in lecithin emulsion using the water-soluble initiator AAPH.

(i) Bulk. The assay was conducted in cobalt-activated stripped corn oil (Eastman Kodak Co., Rochester, NY). Ten milligrams of cobalt chloride hexahydrate (Aldrich Chemical Co.) was pulverized and vortexed in a 25-mL test tube for 10 s. Two hundred microliters of a 2 mg/mL methanolic antioxidant solution (2000 ppm oil basis) and 2 mL of stripped corn oil were added, and the mixture was vortexed for 30 s, then decanted into a 9-cm-diameter covered Petri dish for storage and assay at 95°C. Front-face fluorescence assay of a polyamide plate suspended in the head space was conducted as below for lecithin emulsions. The percent antioxidant effectiveness was assessed at 1 h by the following: $100 \times [(\text{Control RI} - \text{Initial RI}) - (\text{Sample RI} - \text{Initial RI})]/(\text{Control RI} - \text{Initial RI})$. Mean and SD of three trials are usually reported. Three control and three sample plates were measured as defined below under *Lecithin emulsion.*

(ii) Lecithin emulsion. Compounds were also tested in a lecithin emulsion with hematin accelerator. Two hundred milligrams of lecithin (Yelkin DS, Ross and Rowe, Inc.; double bleached, 35% TG) in 10 mL de-ionized (Milli-Q; Waters, Milford, MA) water was sonicated in an ice bath for 15 min at maximum power and sparged for 15 min with a filtered air stream. One milliliter of a 10 mg/mL hematin solution prepared with one drop of 80% NaOH was added. Twenty microliters of a 20 mg/mL methanolic solution of antioxidant was added (400 μg or 2000 ppm, lipid basis). The mixture was vortexed for 30 s and placed in a Petri dish; to the cover of this dish was attached a polyamide-coated terephthalate TLC plate measuring 2×3 cm, coated side facing the vapor space. Samples were incubated at 90°C. Oxidation was monitored by front-face assay of the fluorescent compounds formed by lipid oxidation volatiles on the polyamide plate at *ca.* 30-min intervals. Front-face fluorescence (20) of the polyamide plate was assayed with excitation at 392 nm, and emission was read at both the maximum and the minimum. Detector voltage was 950 V, and all slits were 1 mm. A ratiometric method using Ratiometric Intensity (RI) = Intensity at Maximum (normally at about 470 nm)/Intensity at Minimum was used to minimize fluctuations due to variations of plate position. Percent antioxidant effectiveness was assayed at 2 h and analyzed as described above for bulk oils.

Antioxidant effectiveness of food purees and juices. Lecithin (1.2 g) and 60 mL de-ionized water were mixed and sonicated at full power for 15 min in an ice bath. The emulsion was air-sparged for 15 min, 162 mg AAPH added, and the mixture magnetically stirred for 5 min. The mixture was divided, and to the control half, 30 mL more of de-ionized water was added and the mixture stirred for 5 min more before separating it into three 20-mL portions, each in a separate Petri dish with polyamide plate in the cover as noted above. To the test sample was added 30 mL more de-ionized water and 600 mg of the fruit puree or juice to be assayed, followed by 5 min of stirring and a final division into three 20-mL portions in separate covered Petri dishes. The final composition in each dish was 200 mg lecithin, 27 mg AAPH, and 200 mg fruit in 20 mL de-ionized water. Front-face fluorescence assay differed from the assay for chemical compounds above in that incubation was at 60°C, excitation at 360 nm, and maximum emission at about

420 nm. A 390-nm cutoff filter was used, and the scanning span included the attenuated 360 nm excitation peak, the intensity of which was used to derive the ratiometric RI value as (intensity at maximum)/(intensity of the attenuated 360 nm excitation peak). Detector voltage was lowered to 650 V with 1 mm slits. Analysis was similar to that for chemical compounds.

Antioxidant effectiveness of fruit powders. Assay was conducted in a dispersion of freeze-dried casein-encapsulated fish oil powder (containing 50% TG) with AAPH initiator. Powder (2.4 g) was dispersed in 40 mL of de-ionized water with mechanical shaking to yield a milky suspension, which was divided into two 20-mL portions, one as control and one as test sample. Fruit powder (600 mg) was added to the test sample portion and dispersed with mechanical shaking. A solution of 140 mg AAPH in 40 mL of de-ionized water was divided into 10-mL portions among four Petri dishes. Test sample mixture (10 mL) was added to each of two dishes and 10 mL of control to each of the other two. The final composition of each dish was 600 mg fish oil powder, 35 mg AAPH, and 300 mg fruit powder. Incubation was at 72°C. Front-face fluorescence assay and analysis were similar to those for antioxidant chemical compounds above with excitation at 392 nm and emission at *ca*. 470 nm.

Pyrazine inhibition by antioxidant chemical compounds. An aqueous model system was used comprising 1 M glucose, 3 M glycine, and 1 M NaOH in 10 mL of de-ionized water with or without water-soluble antioxidants at 10 or 1 mol percentage of glycine. The system was designed for small volumes so that small amounts of scarce antioxidants or food samples could be rapidly and repeatedly tested in a microwave system.

(i) Open system. The preceding reactant solution was placed in a 50 mL vial, capped with perforated Saran (S.C. Johnson, Racine, WI) wrap (19) and placed in the center of the rotating carousel microwave. The system was microwaved at full power for 35 s with immediate cold water-cooling by vial immersion for 100 s. A serial schedule was used, with Control (minus antioxidant) heated first and Test Sample (with antioxidant) heated last. This system, where temperature rise is limited by the b.p., was used for most testing of antioxidant compounds.

(ii) Sealed system. The reactant solution was contained in a 20-mL screw-capped scintillation vial, which retained pressure during 30 s of heating. Various methods of placement and schedule were used, such as concurrent measurement at 5 cm from carousel center and 90° between control and sample positions vs. serial measurement with a dummy water-filled vial at 180°. The latter was used for most of the food sample measurements. In such a sealed system, higher temperatures are attained and shorter heating times are used.

Pyrazine inhibition by food powders. The model system used was essentially that outlined above, using the serial system with a water-filled dummy at 180°. Typically, the standard solution (1 M glucose, 3 M glycine. and 1 M NaOH) was diluted 1:3 or 1:4, and 550 mg of the selected food puree or powder or freeze-dried vegetable was added with timed mechanical shaking until dispersed uniformly. Incubation time was 30 s with 60 s cold water-cooling by immersion of the vial.

Pyrazine measurement methods. Pyrazines were collected by SPME and analyzed by GC (24).

(i) SPME. The microwaved sample was equilibrated for 30 min at room temperature after addition of 2 g NaCl. Volatile absorption was carried out by 20-min exposure to the SPME fiber in the headspace with mechanical stirring of the solution.

(ii) GC. The SPME fiber was desorbed in the GC injection port for 2 min at 250°C; the column temperature was 150°C. GC separation was carried out from 150 to 200°C at 4°C/min. 2,5-Dimethyl pyrazine and 2,3,5-trimethyl pyrazine (Aldrich Chemical Co.) were determined based on the retention times of standard compounds. The percent inhibition for each peak was calculated from the integrated GC peak area as 100 × [(Control pyrazine – Sample pyrazine)/Control pyrazine]. When Sample pyrazine exceeds Control, the resulting negative value is plotted as pro-pyrazine activity.

UV/vis spectrophotometry. For assessment of Maillard browning and oxidative browning of polyphenols, optical density was read at 400 nm on a 1:20 dilution of microwaved solutions. Solutions containing polyphenols were titrated to pH 4 to avoid the alkaline enhancement of their spectra. Results were expressed as 100 × (antioxidant/control).

Fluorescence spectrophotometry. Microwaved solutions were diluted 1:200. For both UV/vis and fluorescence spectrophotometry, results were expressed as the ratio of antioxidant to control, as described above. For front-face assay of Maillard browning in potato extrudates, excitation spectra were read at 330, 390, and 440 nm peaks using an emission setting of 482 nm. Ratiometric assay of Maillard browning used the following formula: Ratiometric Browning Index = (Intensity at 390 nm/Intensity at 330 nm) + (Intensity at 440 nm/Intensity at 330 nm). Ratiometry was used to compensate for variations in front-face sample position. For assessment of chlorogenic acid retention in potato extrudates by front-face fluorescence, emission was read at 425 nm from excitation at 330 nm. Details of lipid oxidation assay by polyamide front-face fluorescence are discussed above in antioxidant effectiveness measurement sections.

RESULTS AND DISCUSSION

Antioxidant effectiveness of chemical compounds. Figure 1A shows typical kinetics of polyamide fluorescence development in response to volatiles from oxidation of the lecithin emulsion with and without tannic acid, a prominent polyphenol in mango. There is an obvious induction period (IP) of about 30 min for the control, but the tannic acid sample shows no measurable IP within an appropriate time. Effectiveness of antioxidants in emulsion was therefore estimated at 2 h by the intensity ratio as indicated in the Materials and Methods section. Table 1 shows antioxidant effectiveness in both stripped corn oil (bulk phase) and in a lecithin emulsion of naturally occurring antioxidants of varying potency found in mango. TBHQ and BHA values are included for reference. The contrasting relative effectiveness in bulk vs. emulsion of the analogous gallic acid and protocatechuic acid (bulk > emulsion) with protocatechuic aldehyde (emulsion > bulk) is compatible with the Polar Paradox theory (gallic acid polarity > protocatechuic acid >> protocatechuic aldehyde; 20,26). The strong pro-oxidant activity

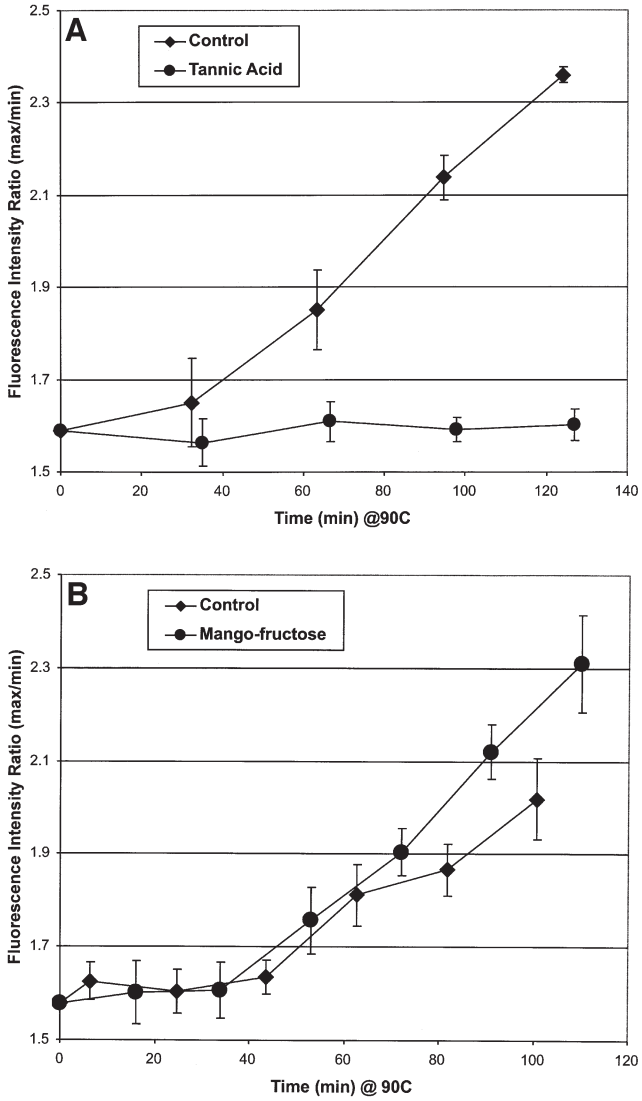

FIG. 1. (A) Tannic acid inhibition (2000 ppm, lipid basis) of oxidation of lecithin emulsion initiated by hematin and (B) mango pro-oxidant activity (200 mg mango-fructose puree/200 mg lecithin) in hematin-initiated oxidizing lecithin emulsion. Both experiments were conducted at 90°C. Volatiles in headspace monitored by ratiometric front-face polyamide fluorescence. Error bars represent SD, $n = 3$.

in emulsion of *p*-coumaric acid is surprising, yet very reproducible. In the hematin system, we have seen pro-oxidant activity for other combinations (mango–fructose, Fig. 1B), which is reversible by EDTA and therefore probably related to metals and reducing agents in a Fenton-type activation of peroxides, often seen with tocopherol at high concentrations. However, *p*-coumaric acid is not a strong antioxidant, even in bulk.

Antioxidant effectiveness of food purees and juices. Figure 2 shows the kinetics of polyamide fluorescence production in the presence and absence of freeze-dried taro. The induction period is less pronounced, but taro antioxidant effectiveness (60%) is clear at 4 h. Table 2 shows effectiveness for plum purees and juices and raisin purees in an AAPH-initiated system. With respect to effectiveness, plum puree > raisin puree > plum juice. When 2.5-fold greater concentrations of the fruits are used, commensurate increases in effectiveness result. The small value for raisin puree and the pro-oxidant activity for plum puree in the hematin system are noteworthy. These are probably related to the similar pro-oxidant activity of *p*-coumaric acid and mango (Table 1, Fig. 1B) and to Fenton-type reduced metal activation of peroxide, since it is not present with AAPH initiator.

TABLE 1
Oxidation Inhibition[a] by Naturally Occurring Phenolic Antioxidants[b] in Hematin-Initiated Lecithin Emulsion (90°C) and in Bulk Corn Oil (95°C)[c]

Antioxidant[d]	Stripped corn oil w/cobalt	Lecithin emulsion w/hematin
TBHQ	86	
Gallic acid	89 ± 18.6	63 ± 31.4
Tannic acid	81 ± 34.2	85 ± 3.38
Protocatechuic aldehyde	49 ± 29.5	83 ± 9.34
Protocatechuic acid	74 ± 25.8	32 ± 8.2
p-Coumaric acid	32 ± 7.95	−164 ± 19
BHA	87 ± 36.1	100 ± 0.64

[a]Values presented as % inhibition, mean ± SD, for $n = 3$, except for TBHQ, which is a reference value.
[b]Antioxidant load for both systems 400 µg, 2000 ppm, lipid basis.
[c]Volatiles in headspace monitored by ratiometric polyamide front-face fluorescence.
[d]Gallic acid, tannic acid, protocatechic aldehyde, protocatechoic acid, and *p*-coumaric acid are commonly found in mango fruits.

Antioxidant effectiveness of food powders. The percent inhibition of oxidation by fruit and beverage powders in AAPH-initiated freeze-dried fish oil emulsions at 72°C were as follows (mean ± SD, $n = 3$): blueberry, 100; grape seed, 100; grape skin, 100; red wine, 80; volatiles in headspace were monitored by ratiometric front-face polyamide fluorescence (300 mg powder/600 mg fish oil). These values reflect the antioxidant effectiveness of fruit and beverage powders in AAPH-initiated freeze-dried fish oil emulsion. The activity of these materials is very high and is undoubtedly related to the concentration advantage of dried powders over purees and juices. The Oxygen Radical Absorbance Capacity (ORAC) values developed by U.S.D.A. Human Nutrition Research Center on Aging (27,28), in an aqueous assay, support these results (units are mmol Trolox equiv/g): prunes 5770, raisins 2830, and blueberries 2400 vs. kale 1770 and broccoli 890.

Pyrazine inhibition by antioxidant compounds. (i) Open system. The pyrazine inhibition capacity of selected compounds is shown in Table 3 for an open system in which temperatures are kept low by the low boiling point of the solution. The potent water-soluble polyphenolic antioxidants gallic acid and propyl gallate show strong activity at 10 mol% glycine, and propyl gallate is active at 1 mol% glycine for both 2,5-dimethyl and 2,3,5-trimethyl pyrazines. Chlorogenic acid shows less activity, but it is still appreciable. Caffeic acid is less active than chlorogenic acid, its quinic acid conjugate, an observation that is consistent with its lower water solubility. The sulfhydryl glutathione and the reductone ascorbic acid both have appreciable activity. Glutathione does not brown by oxidative quinone formation and polymerization, as do the polyphenols, as will be

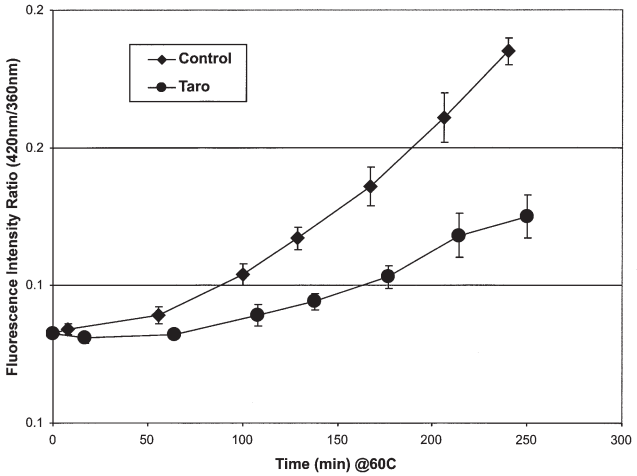

FIG. 2. Inhibition by freeze-dried taro (200 mg taro/200 mg lecithin) of oxidizing lecithin emulsion initiated by 2,2′-azobis-(2-amidinopropane) dihydrochloride at 60°C. Volatiles monitored as in Figure 1. Error bars represent SD, $n = 3$.

discussed shortly. Ascorbic acid browns extensively but inhibits pyrazines. Browning is probably caused by reversion to dehydroascorbic acid, which is known to undergo strong Maillard browning.

(ii) Sealed system. The sealed system, presented in Table 4, behaves like a retort in that no water vapor escape is possible and higher temperatures are reached in the standard reaction time. The system has therefore less reproducibility than the open system, but we have used it for some compounds with high surface activity, such as Trolox, because of the frequent liquid loss from boiling and bumping in the open system. The reduced effectiveness of propyl gallate, glutathione, and ascorbic acid is notable. A prominent feature, although there is large variability, is the pronounced pro-pyrazine activity of ellagic acid, a product often resulting from breakdown of hydrolyzable tannins such as tannic acid. We never found any anti-pyrazine activity for ellagic acid in the microwaved system, in contrast to results found by others in a different model system (7). Reported to be an excellent antioxidant (29), ellagic acid has very low water solubility compared with its parent compound in plants, the highly soluble antioxidant tannic acid. As is shown here, tannic acid is an exceptionally active pyrazine inhibitor.

TABLE 2
Oxidation Inhibition[a] by Fruit Juices and Purees in Either AAPH (A)- or Hematin (H)-Initiated Lecithin Emulsion at 60°C[b]

Fruit juice or puree		Fruit juice or puree	
BHA (A)	34 ± 8	Raisin puree (A)	19 ± 4.3
Plum puree (A) –Trial I	26 ± 4.1	Raisin puree (H)	2 ± 19
Plum puree (A) – Trial II	26 ± 5.2	Plum puree (H)	−9 ± 6.4
Plum Juicy (A) – Trial I	15 ± 7.3	Plum puree[c] (A)	34 ± 7.2
Plum Juicy (A) – Trial II	15 ± 10.9	Raisin puree[c] (A)	43 ±1.3

[a]Values presented as % inhibition, mean ± SD, for $n = 3$.
[b]Conditions: 200 mg puree/200 mg lecithin. Volatiles in headspace monitored by ratiometric front-face polyamide fluorescence.
[c]For these samples, conditions were 500 mg puree/200 mg lecithin.

Maillard-induced browning and attrition of polyphenolic antioxidant compounds. As already noted, we found that polyphenols such as gallic and chlorogenic acids, with totally or partially unsubstituted double bonds in the position *ortho* to the phenol groups, brown extensively under Maillard conditions, presumably when they are oxidized by the free radical precursors of pyrazines. We observed that the quinone–amine adducts formed by such reactions strongly quench the normal Maillard-generated fluorescence. This is shown for gallic acid in Figure 3A, in which our microwaved glucose/glycine model system alone shows moderate browning and strong fluorescence, in contrast to either the heated or unheated system with gallic acid, but without glucose, and thus without Maillard potential. However, when gallic acid is present in the heated model system with glucose and glycine, browning is highly intensified and fluorescence is quenched. Thus, in our high water activity system, the Maillard reaction tends to consume such polyphenols owing to their free radical quenching and thus quinone-forming action. This is graphically illustrated by Figure 3B, in which potato extrudates (30) containing added glucose as a Maillard promoter show strong Maillard fluorescence immediately after extrusion (a high-temperature and quite anaerobic process) as compared with samples containing sucrose and glycerol, which were added to minimize Maillard interaction. After 1–3 wk of aerobic storage at 50°C, however, fluorescence in the glucose-containing Maillard-browned samples declines strongly and color becomes more red-brown. The fluorescence spectra (excitation at 330 nm) of samples stored at 50°C (Fig. 3C) show virtually total loss of chlorogenic acid emission at 425 nm in the stored sample con-

TABLE 3
Percent Inhibitiona of Pyrazine by Antioxidant Compounds in Open Glucose/Glycine System

	2,5-Dimethylpyrazine			2,3,5-Trimethylpyrazine		
Compound	Mean	SD/MD[a]	*n*	Mean	SD/MD[a]	*n*
Gallic acid–10%	87	5.05	4	89.6	3.94	4
Propyl gallate–10%	77.8	NA	1	94.6	NA	1
Propyl gallate–1%	85.3	12.3	3	81.3	10.3	3
Chlorogenic acid–1%	45.4	22.5	4	59.8	17.6	4
Glutathione–1%	79.4	NA	1	53.4	NA	1
Ascorbic acid–1%	52.6	16.9	2	52.2	17.7	2
Caffeic acid–1%				37.9	5.95	3

[a]SD or mean deviation (SD/MD) for replicate number (*n*). NA, not applicable.

TABLE 4
Percent Inhibition[a] of Pyrazine by Antioxidant Compounds in Sealed Glucose/Glycine System

	2,5-Dimethylpyrazine			2,3,5-Trimethylpyrazine		
Compound	Mean	SD[a]	*n*	Mean	SD[a]	*n*
Gallic acid–10%	95.7	NA	1	100	NA	1
Propyl gallate–10%	27.9	NA	1	56.9	NA	1
Glutathione–1%	63.5	NA	1	34	NA	1
Ascorbic acid–1%	38.5	NA	1	34.2	NA	1
Ellagic acid–1%	–167	142	3	–76	87	3
Tannic acid–1%	82	9.4	3	83	11	3
Trolox–1%	66.8	17	3	67.5	16.4	3

[a]SD or mean deviation (SD/MD) for replicate number (*n*). NA, not applicable.

taining added glucose, compared with the virtually unchanged spectra for the control and for the sucrose- and glycerol-containing extrudates. The spectra at zero storage (Fig. 3D) show no such loss in the glucose sample.

The Trolox anomaly. The synthetic monophenol Trolox was tested because it is a water-soluble analog of tocopherol. This compound is unusual in that it is a potent water-soluble antioxidant that is totally ring-substituted, so it cannot form

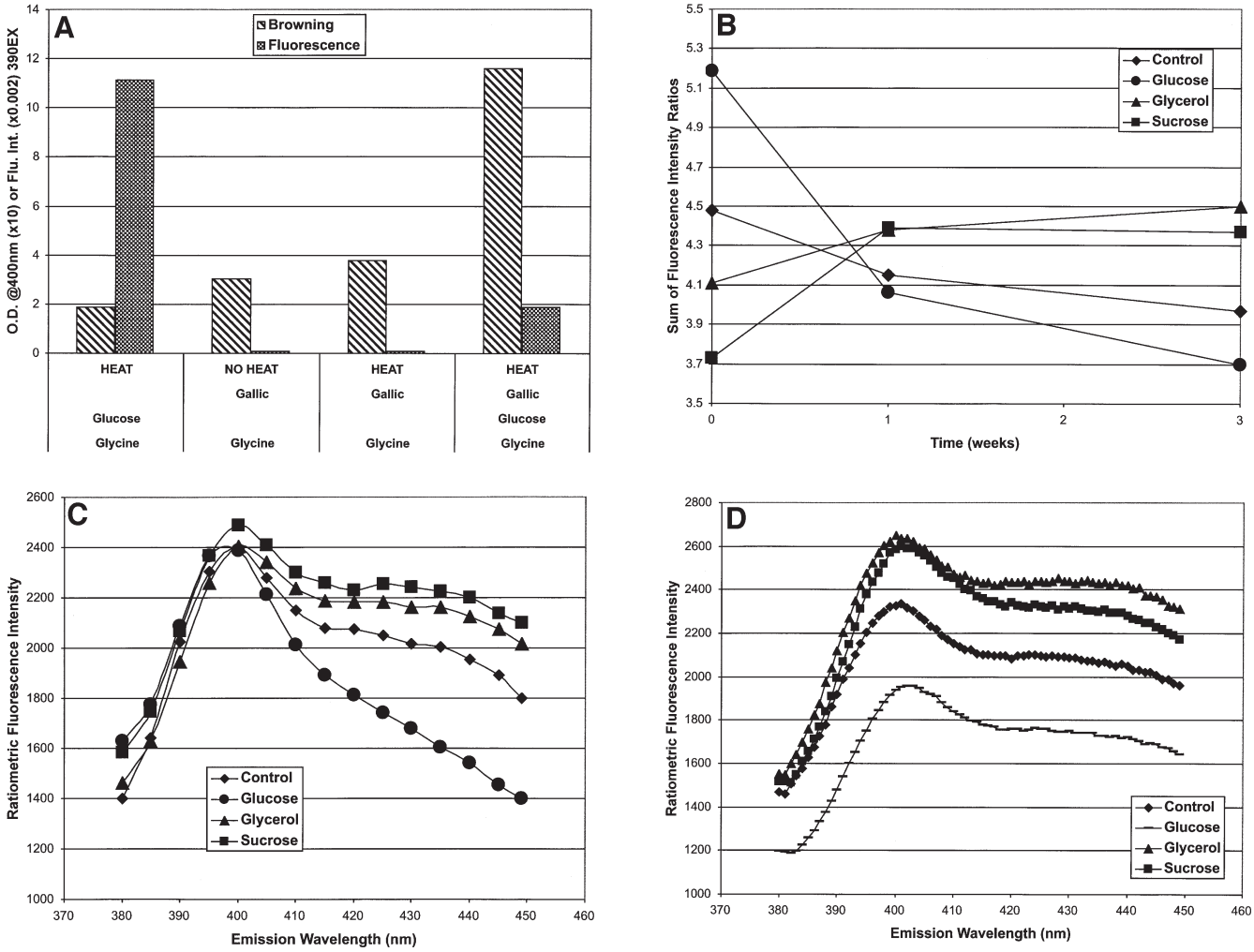

FIG. 3. (A) Effect of gallic acid on browning and fluorescence. Abbreviations: O.D. = optical density at 400 nm, a measure of browning. Flu. Int. = fluorescence intensity of microwaved solution arising from Maillard browning. (B) Front-face fluorescence intensity (sum of low and high excitation wavelengths' intensity ratios from 482 nm emission) of potato extrudates at 0, 1, and 3 wk storage at 50°C. (C) Front-face fluorescence emission intensity of potato extrudates stored 1 wk at 50°C. Excitation was at 330 nm, which is specific for chlorogenic acid emitting at ~425 nm. (D) Front-face fluorescence emission intensity of potato extrudates prior to storage. Excitation as in (C).

TABLE 5
Variable Trolox Effect on Inhibition of Pyrazine Production, Browning (O.D. at 400 nm) and Maillard Fluorescence Emission Intensity (excitation 390 nm) Resulting from Planned Alterations in Sample Positioning in the Microwave

Trolox trial	Pyrazine production Tro/Cont	Browning Tro/Cont	Fluorescence Tro/Cont
2	0.37	0.79	
3	0.62	0.875	0.91
4	0.5	0.59	0.61
5	0.26	0.5	0.49
7	0.063	0.21	0.35
8	0.27	0.31	0.41
9	0.14	0.38	
10	0.28	0.4	
11	0.49	0.72	
G1[a]	0.21	0.46	
G2[a]	0.37	0.42	

[a]Glutathione data shown for comparison. Tro/Cont, Trolox effect divided by Control effect.

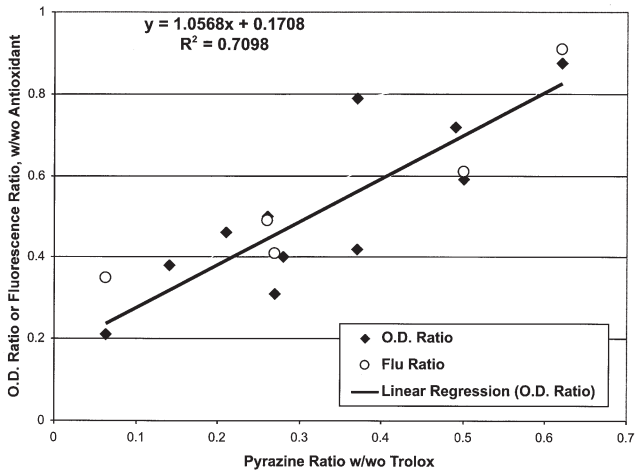

FIG. 4. Correlation of pyrazine inhibition ratio with browning and fluorescence ratios with and without Trolox. Equation calculated as linear regression of browning inhibition ratio on pyrazine ratio.

quinone–amine adducts, which, as stated earlier, polymerize, brown intensely, and quench fluorescence. Table 5 and Figure 4 show that, unlike the polyphenols, variable pyrazine inhibition by Trolox (variations due to altering geometry of placement in the microwave) correlates with reduction of both browning and fluorescence. We have tried to duplicate this behavior with natural, non-quinone-forming antioxidants such as ferulic or sinapic acid, but solubility problems interfere. In a different system using the presumably non-quinone-forming or sterically hindered antioxidants ellagic, ferulic, and syringic acids, Djilas and Milić (7) state, without showing data, that "color intensity decreased with increasing concentration and degree of oxidation of the phenolic compounds."

Effect of pH. It could be claimed for the phenolic acids tested in this work that pyrazine inhibition might be partially due to pH lowering by phenolic acids in the model system (31). Table 6 shows pH comparisons between the initial and heated solutions for glucose/glycine systems containing gallic acid, Trolox, or controls without antioxidant. The pH values observed are nowhere near Maillard inhibitory levels, and the expected small drop due to the Maillard reaction in the antioxidant systems was quite similar to that in the control systems. It seems clear that the pyrazine inhibition we observed was not induced by pH changes.

Pyrazine inhibition by food and beverage components. Table 7 shows results of tests on pyrazine inhibition by spices, beverages, and freeze-dried vegetable powders. The strong pro-pyrazine activity of unsteeped plum puree is probably due to high initial concentrations of pyrazines produced by Maillard reactions during high-temperature drying. Alternatively, high sugar concentrations in dried plum may dictate that Maillard browning produces so much pyrazine that the plum antioxidants, though in high concentration, cannot inhibit its formation. Taro also shows pro-pyrazine activity, which may be due to the high starch concentration of taro and some hydrolysis to reducing sugars. Freeze-dried vegetables, in general, show pro-pyrazine activity. Among the spices, cloves; rosmarinic acid; and fresh, but not aged, commercially produced water-soluble antioxidant extract rosemary powder show pronounced pyrazine inhibition whereas the beverages black and green tea, known for their antioxidant theaflavins and thearubigens (black) and catechin gallates (green), are strongly inhibitory.

Table 8 shows final results on pyrazine inhibition by a number of fruit powder components. Among the fruit powders, grape seed, grape skin, fresh but not aged cranberry, and red wine are highly inhibitory, consistent with their ORAC values. Steeped blueberry powder also shows noticeably greater inhibition than the unsteeped material. Partially confirming the initial result in Table 7, steeped dried plum puree and freeze-dried taro powder promote 2,5-dimethyl pyrazine but inhibit 2,3,5-trimethyl pyrazine.

The inhibitory effects of antioxidants on pyrazine formation can be summarized as follows: (i) The effect depends on high concentrations of water-soluble antioxidants, concentrations usually 100-fold greater than required for lipid oxidation inhibition; (ii) such antioxidants occur naturally in some foods and are usually polyphenolic quinone formers; (iii) in retarding pyrazine formation, oxidized polyphenolic antioxidants may form quinone–amine polymers, which highly enhance browning and attendant stiffening and quench Maillard fluorescence; (iv) thus, in the final oxidative phase of the Maillard reaction, naturally occurring polyphenolic antioxidants and reducing agents such as glutathione and ascorbic acid are partially consumed, with some unavoidable nutritional loss; (v) monophenols,

TABLE 6
pH of Model Systems Before and After Heating

System	Concentration (mol% glycine)	Initial pH	Heated pH
Gallic acid (GA)	10	8.24	7.92
Control for GA		8.90	8.58
Trolox (TR)	1	8.53	8.34
Control for TR		8.67	8.28

TABLE 7
Percent Inhibition of Pyrazine by Dried Vegetables, Spices, and Beverages[a]

	2,5-Dimethylpyrazine			2,3,5-Trimethylpyrazine		
Substance	Mean	SD/MD[b]	*n*	Mean	SD/MD[b]	*n*
Plum juicy, dried plum powder	–158	176	4	–39	69	4
Freeze-dried taro	–234	314	6	–99	199	6
Freeze-dried kale, not steeped	–113	39	2	–395	175	2
Freeze-dried kale, steeped	–95	15	2	–226	1	2
Freeze-dried broccoli	–61.3	16.2	3	–28.7	24	3
Ground cloves	52	8	2	100	0	2
Ground oregano	–193	7	2	59	24	2
Herbalox rosemary seasoning, aged	–20.5	36.1	2	8.2	18	2
Herbalox rosemary seasoning, fresh	41	18.4	2	100	0	2
Rosmarinic acid	100	0	2	100	0	2
Black tea infusion	100	0	2	100	0	2
Green tea infusion	92	15	3	100	0	3

[a]Load 550 mg (except 1.01 g for tea) in sealed glucose/glycine system at 1:4 dilution of standard.
[b]SD or mean deviation (SD/MD) for number of replicates (n).

TABLE 8
Percent Inhibition of Pyrazine by Fruit Purees and Dried Powders[a]

	2,5-Dimethylpyrazine			2,3,5-Trimethylpyrazine		
Substance	Mean	SD/MD[b]	*n*	Mean	SD/MD	*n*
Wild blueberry, not steeped	23.5	16.3	4	74	1.41	4
Wild blueberry, steeped	86	4.24	4	85	1.41	4
Grape seed	100	0	2	100	0	2
Grapeskin	100	0	2	100	0	2
Red wine	100	0	2	100	0	2
Cranberry, aged	29	NA	1	43	NA	1
Cranberry, new sample	100	NA	1	100	NA1	1
Dried plum puree, steeped	–83.5	13.4	2	32.5	7.82	2
Taro, freeze-dried	–7	NA	1	29	NA	1

[a]Load of 560 mg in sealed glucose/glycine system at 1:4 dilution of standard.
[b]SD or mean deviation (SD/MD) for number of replicates (n).

which do not form quinines in oxidation, appear to inhibit pyrazines with less attendant browning and fluorescence; (vi) powders of grape seed, grape skin, and red wine are highly pyrazine-inhibitory, steeped blueberry is strongly so, but plum purees are moderately pro-pyrazine, and the freeze-dried vegetables tested are strongly pro-pyrazine; (vii) black and green tea infusions are highly inhibitory, whereas spices have mixed effects.

The pro-pyrazine effects of plum purees are in sharp contrast to their very high ORAC values (cited earlier) and our oxidation inhibition results. Pyrazines that are preformed in heat drying, or overwhelming concentrations of reducing sugars, may be the cause.

There remains the anomaly that added antioxidants and reducing agents of diverse types inhibit pyrazines, although the Maillard reaction, in addition to pyrazine formation, is considered to produce reductones and melanoidins with reducing power (3,31).

In using carbohydrate module labeling and HPLC/MS of the products from a heated glucose/glycine model, Totlani and Peterson (6) presented evidence for adducts of epicatechin and glucose fragments, for which, among other mechanisms, they suggest a phenol-carbonyl-type, Maillard-inhibitory condensation, which they state has been previously shown in wine. This is a polar ionic reaction. In contrast, our results in a microwaved system appear to support a free radical mechanism. Thus, reducing agents other than polyphenols, such as glutathione, ascorbic acid, and Trolox, which cannot form phenol-carbonyl-type adducts, inhibit pyrazines. In addition, during inhibition of pyrazines, polyphenolic antioxidants show evidence of oxidative destruction (loss of polyphenol fluorescence, quenching of Maillard fluorescence, and strong browning intensification).

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