Influence of Cherry Tissue on Lipid Oxidation and Heterocyclic Aromatic Amine Formation in Ground Beef Patties

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The effects of tart cherry tissue added at an 11.5% level on the oxidation of lipids in raw and cooked ground beef patties and on the formation of heterocyclic aromatic amines (HAAs) in the fried patties were investigated. Oxidation was significantly influenced by the addition of tissue from two varieties of tart cherries, Montmorency and Balaton. Thiobarbituric acid-reactive substances values for raw and cooked ground beef patties containing cherry tissue were significantly ($p < 0.05$) smaller than those for the control beef patties. Cholesterol oxidation was also influenced by the presence of cherry tissue. After 4 days of refrigerated storage, cholesterol oxides represented 5.2% of the total cholesterol content of cooked control beef patties and 2.0 and 1.7% of the total cholesterol in patties containing Montmorency and Balaton cherry tissue, respectively. The formation of mutagenic/carcinogenic HAAs during frying of the patties was inhibited by components in the cherry tissue. The concentrations of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the principal HAA in cooked muscle foods, were reduced 93 and 87% by Montmorency and Balaton cherry tissue, respectively. Tart cherry tissue may represent a new approach to reducing HAA formation in cooked beef patties as well as being a natural source of antioxidants to delay the rapid onset of lipid and cholesterol oxidation in meat products.

Keywords: *Ground beef; cherry; heterocyclic aromatic amines; cholesterol oxidation products; lipid oxidation*

INTRODUCTION

Lipid oxidation is one of the primary mechanisms of quality deterioration in cooked meat products during storage (Kanner, 1994). Off-flavor development in meat has been attributed to the formation of a complex mixture of carbonyl compounds during the autoxidation of unsaturated fatty acids (Drumm and Spanier, 1991). Oxidation products also adversely affect the color, texture, nutritive value (Pearson et al., 1983), and even safety of meats (Addis, 1986). Cholesterol also readily undergoes oxidation in meat products during and after cooking (Monahan et al., 1992). Recently, interest in the possible toxicological effects of lipid oxidation products, particularly cholesterol oxidation products (COPs), has increased. In vivo free radical-mediated lipid peroxidation has been implicated in a variety of pathological processes including inflammation and rheumatoid arthritis (Halliwell and Gutteridge, 1985), atherosclerosis (Quinn et al., 1987), and mutagenesis and carcinogenesis (Ames et al., 1982; O'Brien, 1982). The influence of dietary lipid oxidation products on these processes is largely speculative, although animal studies do provide some compelling evidence implicating certain COPs as initiators of atherosclerotic lesions in blood vessels (Peng et al., 1985). Oxides of cholesterol have been detected in a variety of processed foods including meat, egg, and dairy products [reviewed by Paniangvait et al. (1995)]. Many questions, however, remain to be answered before any firm conclusions can be drawn regarding the atherogenicity of COPs in the diet (Paniangvait et al., 1995).

Heterocyclic aromatic amines (HAAs) are dietary compounds that are formed naturally during cooking of muscle foods (Skog, 1993). The most commonly reported HAAs in meats are 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). At least 17 HAAs have been isolated from and identified in foods and model systems (Johansson et al., 1993). These compounds are mutagenic in the Ames/*Salmonella* assay (Sugimura et al., 1988), are carcinogenic in rodents and monkeys (Adamson et al., 1990; Wakabayashi et al., 1993), and are suspected to be carcinogenic in humans (IARC, 1993). The HAAs are thought to be formed during the cooking process through reactions involving creatine or creatinine, sugars, and amino acids, all common components of muscle tissue (Skog, 1993).

These undesirable reactions in meats can be controlled in part by the addition of antioxidants. For example, supranutritional dietary supplementation of vitamin E for the subsequent benefit of increased lipid stability in animal food products has been extensively reported for poultry, beef cattle, veal calves, and pigs (Mielche and Bertelsen, 1994). Similarly, many studies have addressed the efficacy of adding antioxidants to meat during processing for control of lipid oxidation in processed and cooked meats. These compounds can range from commercial phenolic antioxidants to more exotic compounds isolated from plant products. The

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applications of antioxidants in meat systems have been described in recent reviews by Gray and Crackel (1994) and Mielche and Bertelsen (1994). More recently, the inhibition of HAA formation in fried ground beef patties by vitamin E when added directly to ground beef or applied to the surface of the patties was reported by Balogh et al. (1995).

The antioxidant activity of cherry tissue in combination with spices and oat bran has been demonstrated in uncooked low-fat (10%) ground beef patties during refrigerated and frozen storage (Liu et al., 1995). Furthermore, cherry fractions containing phenolic components such as isoflavones and anthocyanins have shown considerable response in a fluorescence-based model system developed for the rapid evaluation of compounds with potential antioxidant activity (Strasburg et al., 1998). The objectives of this study were to further evaluate the potential of tissues from two tart cherry varieties (Montmorency and Balaton) as inhibitors of lipid oxidation in raw and cooked ground beef patties and as inhibitors of HAA formation in cooked patties.

MATERIALS AND METHODS

Safety. HAAs are mutagenic/carcinogenic and must be handled with appropriate safety precautions.

Reagents. Cholesterol (cholest-5-en-3β-ol) and 6-ketocholestanol (6-oxo-5-cholestan-3β-ol) standards were purchased from Sigma Chemical Co. (St. Louis, MO). Cholesterol oxide standards, α-and β-epoxides (5α,6α-epoxycholestan-3β-ol and 5β,6β-epoxycholestan-3β-ol), 7α- and 7β-hydroxycholesterols (5-cholestene-3β,7α-diol and 5-cholestene-3β,7β-diol), 7-ketocholesterol (7-oxo-5-cholesten-3β-ol), 20α-hydroxycholesterol (5-cholestene-3β,20α-diol), 25-hydroxycholesterol (5-cholestene-3β,25-diol), and cholestane-3β,5α,6β-triol were obtained from Steraloids Inc. (Wilton,NH). Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA plus 1% TMCS) was purchased from Pierce Chemical Co. (Rockford, IL). HAA standards (IQ, MeIQ, MeIQx, DiMeIQx, and PhIP) were purchased from Toronto Research Chemicals, Toronto, Canada. The HAA standard [FEMA (Flavor and Extracts Manufacturer's Association)] and the internal standard, caffeine, were gifts from Dr. Mark Knize, Lawrence Livermore National Laboratory, Livermore, CA. The FEMA standard contained IQ, MeIQ, MeIQx, DiMeIQs, and PhIP, each at 5 ng/μL. A propylsulfonic acid (PRS) Bond-Elut column (500 mg) and C18 cartridges (100 mg) were purchased from Varian, Inc., Harbor City, CA. Extrelut-20 columns and Extrelut refill diatomaceous earth were obtained from E. M. Separations Technology, Gibbstown, NJ. All other chemicals were of analytical grade and were obtained from Fisher Scientific, Fair Lawn, NJ. Standard laboratory safety practices such as the use of goggles and latex gloves for handling chemicals and potential hazards were followed. All extractions were carried out in efficient fume hoods.

Materials. Freshly ground beef with 15% fat was purchased from a local commercial meat company and used within 1 h of purchase. Frozen, pitted Montmorency and Balaton tart cherries were obtained from commercial growers through the Cherry Marketing Institute, Inc. (Dewitt, MI). The cherries were flushed with nitrogen in freezer bags prior to their storage at −20° C until ready to use.

Preparation of Ground Beef Patties. Patties were prepared from ground beef as follows: control patties (no ingredients added) and patties containing 11.5% tart cherry tissue (Montmorency and Balaton). Cherries were flaked through an Urshel comitrol (Urshel Laboratories, Inc., Valparaiso, IN) using a 3-K-030240-V head. Patties were prepared by mixing the flaked cherry tissue with the ground beef by hand and then mixing in a Keebler mixer (Keebler Inc., Chicago, IL) for 1 min. Each patty was formed by weighing 100 g in a Petri dish (9 cm × 1.5 cm) to ensure patty uniformity. Patties were vacuum packaged and stored at −20 °C until ready to use. The study was repeated three times with three different batches of ground beef with fat contents of 15 ± 1%.

Sample Preparation. *Refrigerated Study.* The patties were thawed overnight in a cooler at 4 °C, then placed on polystyrene trays, wrapped with a retail commercial oxygen-permeable PVC stretch overwrap having an oxygen transmission rate of 6000−8000 cm^3 O_2 m^{-3} 24 h^{-1}, and stored at 4 °C under fluorescent light for 9 days. Samples were taken immediately for fat and moisture determinations and every 3 days for lipid oxidation measurements.

Cooked Study. Patties were fried in a Teflon-coated electric frying pan set at 170 °C for 8 min per side, total cooking time of 16 min. Final internal temperature was 62 ± 3 °C. The weights of the patties were recorded before and after cooking. The cooked patties were placed on polystyrene trays, wrapped with retail commercial oxygen-permeable PVC stretch overwrap, and stored at 4 °C in the dark for 4 days. Samples were taken immediately after cooking for cholesterol determination and at 2 day intervals for lipid and cholesterol oxidation measurements.

To generate HAA formation in the cooked patties, two cooking procedures were investigated. Initially, the patties were fried in the electric frying pan set at 170 °C for 8 min per side, total cooking time of 16 min. Final internal temperature was 62 ± 3 °C. Results indicated very little HAA formation using this cooking protocol. The patties were subsequently fried in a Teflon-coated electric frying pan set at 225 °C for 10 min per side, total cooking time of 20 min. Final internal temperature was 68 ± 3 °C. This time/temperature combination permitted the formation of measurable concentrations of HAAs and facilitated the investigation of the inhibiting activity of cherry tissue.

Analyses. Cooking yield percentages were determined using two patties from each replication and were calculated as (cooked patty weight/raw patty weight) × 100. Fat and moisture contents were determined according to the respective AOAC methods (1992). Lipid oxidation was assessed in the raw and cooked ground beef patties by the 2-thiobarbituric acid method of Tarladgis et al. (1960), as modified by Crackel et al. (1988). Two patties for each treatment within each repeated experiment were analyzed. Thiobarbituric acid-reactive substances (TBARS) values were expressed as milligrams of malonaldehyde equivalents per kilogram of meat.

Total lipids for cholesterol oxide analyses were extracted from cooked samples (5 g) according to the dry column procedure of Marmer and Maxwell (1981).

Cholesterol oxidation products (COPs) in the lipid extracts were isolated according to a solid-phase extraction procedure, using 3 mL Superclean LC-Si SEP tubes (Supelco, Bellefonte, PA) filled with 300 mg of silica packing (40 μm particles, 60 Å pores). The COPs were derivatized to their trimethylsilyl (TMS) ether derivatives using BSTFA/TMCS reagent and quantitated by capillary gas chromatography (Monahan et al., 1992). This was achieved using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and a DB-1 column (15 m × 0.25 mm i.d, 0.25 μm film thickness, J&W Scientific, Folsom, CA). Oven temperature programming was as follows: 170−220 °C at 10 °C/min, 220−236 °C at 0.4 °C/min; 236−325 °C at 10 °C/min; held isothermally at 350 °C for 25 min. Injector port and detector temperatures were 275 and 330 °C, respectively. TMS derivatives of the COPs were injected on the column with a split ratio of 11:1. The identification of COPs in the meat samples were based on comparison of the retention times of sample TMS derivatives relative to those of the standards COPs. Recovery studies on COPs were performed with various concentrations of COPs standards and the internal standard (6-ketocholestanol) as described by Lai et al. (1995). The percent recoveries ranged from 82 to 89%.

Cholesterol was extracted from the cooked patties using the direct saponification procedure of Adams et al. (1986). The cholesterol content of underivatized cholesterol extracts in

Table 1. Proximate Composition and Cooking Yield of Raw Ground Beef Patties[a,b]

treatment	moisture, %	fat, %	cooking yield, %
control	64.4 ± 0.15	14.3 ± 0.20	56.3 ± 0.10
Montmorency	67.7 ± 0.28	12.9 ± 0.18	51.6 ± 0.16
Balaton	65.8 ± 0.32	12.3 ± 0.09	49.6 ± 0.18

[a] Montmorency and Balaton cherry tissues were added to ground beef patties at the 11.5% level. [b] Data represent the mean and standard deviation of duplicate analyses of two patties for each treatment in three replications.

dimethylformamide was determined by gas chromatography with flame ionization detection and the DB-1 column as described above.

Concentrations of HAAs in fried patties were determined according to the standard addition method of Gross and Grüter (1992). Because of the time involved with this procedure, HAA concentrations were determined for only the third of the three repeated experiments. Balogh et al. (1995) demonstrated a range of HAA concentrations in different batches of patties fried under similar conditions. However, the effects of temperature and antioxidants were consistent among the various repeated experiments. For each analysis (repeated five times), two fried patties were blended and four subsamples were extracted. To determine extraction recoveries for each HAA, two of the four subsamples were spiked with 250 ng of each HAA (IQ, MeIQ, MeIQx, DiMeIQx, and PhIP) dissolved in methanol. Separation of the HAAs was carried out on a TSK-gel ODS80-TM column (4.6 mm i.d. × 25 cm, Tosoh Haas, Montgomeryville, PA). The flow rate of the mobile phase was 1 mL/min. The initial ratio of acetonitrile/buffer (triethylamine phosphate, 0.01 M, pH 3.2) was 8:92, which increased to 17:83 during the first 10 min. Acetonitrile concentration continued to increase until the ratio was 25:75 in the next 10 min and then 55:45 in the next 10 min. Over the next 5 min, the acetonitrile/buffer ratio increased to 80:20. After 35 min, the ratio returned to its original 8:92 for 10 min to allow the column to reequilibrate before the next injection. Samples were analyzed on a Millennium 2010 HPLC system (Millipore Corp., Milford, MA) with a photodiode array detector (model 991) and a scanning fluorescence detector (model 474).

For statistical analysis, all data were analyzed by analysis of variance (ANOVA), and means were separated by Duncan's multiple-range test, using a statistical computer program (MSTAT-C) developed at Michigan State University.

RESULTS AND DISCUSSION

Proximate Composition. The fat and moisture contents and cooking yields of the three patty treatments are summarized in Table 1. The fat contents of the cherry patties were, as expected, lower than that of the control patties, whereas the moisture contents were greater. The addition of the cherry tissue also decreased the cooking yield, which is directly related to the higher moisture contents of these patties.

Lipid Oxidation in Raw and Cooked Patties. Analysis of variance of lipid oxidation data for raw and cooked patties revealed that the TBARS values were significantly ($p < 0.05$) influenced by the addition of tart cherry tissue, but not by the variety of tart cherry added. The extent of lipid oxidation was significantly greater ($p < 0.05$) in raw control patties after 3, 6, and 9 days of storage at 4 °C (Table 2) when compared to the patties containing the added cherry tissue. Initially, all patties had similar TBARS values. The TBARS values of the control patties increased steadily during the first 6 days of storage. However, the patties with cherries underwent very little oxidation during the storage period. There was no significant difference between the two tart varieties in terms of controlling lipid oxidation. The

Table 2. TBARS Values of Raw Ground Beef Patties Refrigerated at 4 °C for 9 Days[a,b]

	mg of malonaldehyde equivalents/kg of meat			
treatment	day 0	day 3	day 6	day 9
control	0.35 ± 0.06[a]	1.86 ± 0.13[a]	2.28 ± 0.13[a]	2.19 ± 0.06[a]
Montmorency	0.33 ± 0.04[a]	0.46 ± 0.09[b]	0.40 ± 0.05[b]	0.48 ± 0.05[b]
Balaton	0.34 ± 0.08[a]	0.40 ± 0.04[b]	0.46 ± 0.03[b]	0.62 ± 0.18[b]

[a] Data represent the mean and standard deviation of duplicate analyses of two patties for each treatment in three replications. [b] Means in the same columns bearing different superscripts are significantly different ($p < 0.05$).

Table 3. TBARS Values of Cooked Ground Beef Patties Stored at 4 °C for 4 Days[a,b]

	mg of malonaldehyde equivalents/ kg of meat		
rreatment	day 0	day 2	day 4
control	1.78 ± 0.38[a]	5.63 ± 1.12[a]	8.83 ± 1.44[a]
Montmorency	0.47 ± 0.09[b]	0.84 ± 0.09[b]	1.35 ± 0.23[b]
Balaton	0.52 ± 0.13[b]	0.92 ± 0.23[b]	1.12 ± 0.27[b]

[a] Data represent the mean and standard deviation of duplicate analyses of two patties for each treatment in three replications. [b] Means in the same columns bearing different superscripts are significantly different ($p < 0.05$).

cooked ground beef patties oxidized rapidly over the 4-day storage period, particularly over the first 2 days (Table 3). The extent of lipid oxidation was significantly greater ($p < 0.05$) in the control patties after 2 and 4 days of storage compared to the cherry patties. The addition of cherry tissue to ground beef before cooking significantly ($p < 0.05$) delayed lipid oxidation in the cooked products.

These results are quite similar to those reported by Liu et al. (1995) and clearly demonstrate that cherry tissue effectively inhibits the development of oxidative rancidity in both raw and cooked ground beef patties during storage. The antioxidant mechanism of cherry tissue is not completely understood, but recent studies indicate that anthocyanin components have significant antioxidant activity.

Strasburg et al. (1998), using a fluorescence-based liposome assay, demonstrated that the antioxidant activity of anthocyanins isolated from Montmorency and Balaton tart cherries was comparable to those of butylated hydroxyanisole, butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone. Furthermore, three anthocyanins isolated from cherry tissue each showed activity comparable to those of BHT and propyl gallate and superior to that of α-tocopherol. These results suggest that whole cherries or cherry extracts may be excellent sources of antioxidants for food applications.

Cholesterol Oxidation in Cooked Ground Beef Patties. The mean cholesterol contents of the patties immediately after cooking were 0.83 ± 0.06, 0.74 ± 0.04, and 0.74 ± 0.04 mg of cholesterol/g of cooked patty for the control patties and those containing Montmorency and Balaton cherries, respectively. Pie et al. (1991) reported a cholesterol value of 0.83 mg/g of minced beef cooked for 6 min and 1 mg/g of minced beef cooked for 20 min. Cholesterol oxidation is initiated by abstraction of the allylic C-7 hydrogen, with the subsequent formation of C-7 COPs such as 7α- and 7β-hydroxycholesterols and 7-ketocholesterol (Smith, 1981). Many investigators have claimed C-7 COPs as the predominant cholesterol oxides in beef (Park and Addis, 1987; Paniangvait et al., 1995). In general, only three COPs

Table 4. Concentrations of Cholesterol Oxidation Products (Micrograms per Gram of Meat) in Cooked Ground Beef Patties Stored at 4 °C for 4 Days[a,b]

	day 0			day 2			day 4		
treatment	7α-OH	7β-OH	7-keto	7α-OH	7β-OH	7-keto	7α-OH	7β-OH	7-keto
control	2.50 ± 0.19^{a}	1.80 ± 0.34^{a}	3.15 ± 0.2^{a}	12.60 ± 1.00^{a}	6.10 ± 0.80^{a}	17.20 ± 1.00^{a}	17.90 ± 1.10^{a}	9.40 ± 0.09^{a}	16.00 ± 1.00^{a}
Montmorency	0.87 ± 0.06^{b}	1.10 ± 0.01^{ab}	1.70 ± 0.13^{c}	1.73 ± 0.14^{b}	3.20 ± 0.18^{b}	2.90 ± 0.15^{b}	6.03 ± 0.90^{b}	5.50 ± 0.08^{bc}	3.40 ± 0.30^{c}
Balaton	0.93 ± 0.05^{b}	1.45 ± 0.09^{a}	2.07 ± 0.12^{b}	1.07 ± 0.08^{b}	3.10 ± 0.15^{b}	3.70 ± 0.20^{b}	1.40 ± 0.13^{c}	6.50 ± 0.30^{abc}	4.50 ± 0.35^{b}

[a] Data represent the mean and standard deviations of duplicate analyses of two patties for each treatment in three replications. [b] Means in the same columns bearing different superscripts are significantly different ($p < 0.05$).

were consistently present in detectable quantities in all ground beef samples, cholest-5-ene-3β,7α-diol (7α-OH), cholest-5-ene-3β,7β-diol (7β-OH), and 7-oxocholest-5-en-3β-ol (7-keto). The formation of COPs in cooked ground beef patties was significantly ($p < 0.05$) influenced by the addition of tart cherry tissue but not by the variety added. On day 0, that is, immediately after cooking, detectable amounts of these COPs were found in all samples (Table 4). However, the patties containing the added cherry tissue contained significantly ($p < 0.05$) smaller concentrations of 7α-OH, 7β-OH, and 7-keto than did the control patties. The concentrations of these COPs in the control patties increased significantly ($p < 0.05$) over the 4 day storage period. The concentrations of COPs in the cherry patties also increased. However, the concentrations of the individual COPs were significantly ($p < 0.05$) smaller than those of the control patties. There was no significant difference after 2 and 4 days of storage in the 7α-OH, 7β-OH, and 7-keto concentrations in the patties prepared with the tart cherry tissues. Total COPs concentrations in the control patties and in Montmorency and Balaton patties are summarized in Figure 1a. These data indicate that Montmorency and Balaton cherry tissue inhibited COPs formation by 50 and 40%, respectively, when the patties were analyzed immediately after cooking. After 2 days of storage, both Montmorency and Balaton cherry patties contained 78% less COPs than controls. After 4 days, these values were 66 and 71%, respectively.

Total cholesterol oxide concentrations expressed as percentages of the cholesterol content in cooked meat are presented in Figure 1b. The values at day 0 were slightly higher than those reported by other investigators (Park and Addis, 1987; Pie et al., 1990). After 2 days of refrigerated storage, the total COPs present in the cooked control patties represented 4.3% of the total cholesterol. However, in the case of both Montmorency and Balaton patties, total COPs accounted for 1.0% of the total cholesterol. After 4 days of storage, the percentage of total COPS to cholesterol was much higher in the control patties (5.2%) compared to the Montmorency (2.0%) and Balaton (1.7%) patties. These results again demonstrate the inhibitory effect of tart cherry tissue on the formation of COPs in ground beef patties after cooking and during storage.

Relationship between Lipid and Cholesterol Oxidation. The data for lipid oxidation indicated a significant linear correlation ($r = 0.94$) between TBARS values and COPs formation in cooked ground beef patties stored over 4 days. The correlation coefficients for 7α-OH, 7β-OH, and 7-keto concentrations with TBARS values were 0.95, 0.62, and 0.88, respectively. Cholesterol, being an unsaturated lipid, will undergo oxidation via free radical processes in the same manner as polyunsaturated fatty acids (PUFAs) and their esters. Any events forming free radicals are likely to initiate cholesterol oxidation (Smith, 1981). As PUFAs are likely to be more susceptible to oxidation than cholesterol, Smith (1981) suggested that hydroperoxides of PUFAs might effect the oxidation of cholesterol present in the meat muscle tissue. The relationship between off-flavor development (as measured by TBARS values) and cholesterol oxidation in meats has been studied by several groups. Park and Addis (1987) detected oxidation products in the low parts per million range in broiled beef steaks but not in precooked beef products. As rancidity developed in cooked meats during storage, oxidation of cholesterol became more apparent. De Vore (1988) reported similar trends for raw and cooked ground beef and established correlation coefficients of 0.82 and 0.98 between TBARS values and 7-ketocholesterol concentrations for raw and cooked ground beef, respectively. Similarly, Monahan et al. (1992) demon-

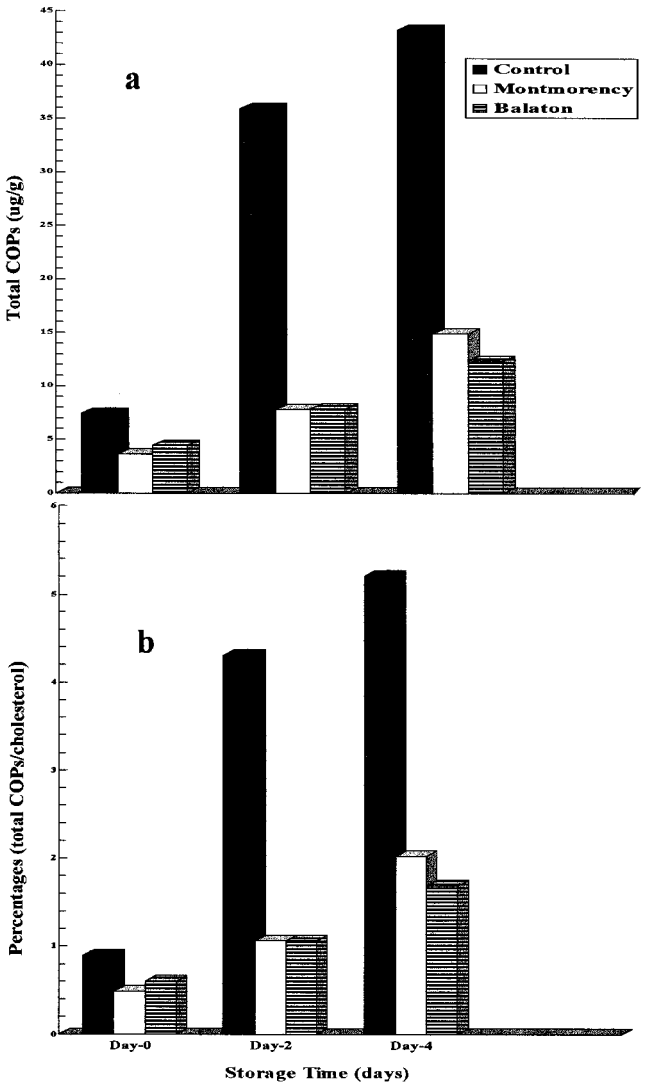

Figure 1. Total concentration (μg/g) of cholesterol oxidation products (a) and percentage of total cholesterol oxides/cholesterol (b) in cooked ground beef stored at 4 °C for 4 days.

Table 5. Heterocyclic Aromatic Amine Content of Fried Ground Beef Patties (Nanograms per Gram)[a,b]

	IQ	MeIQ	MeIQx	DiMeIQx	PhIP	total HAAs
control	1.1 ± 0.7[a]	0.6 ± 0.2[a]	9.2 ± 4.4[a]	2.7 ± 1.2[a]	12.0 ± 2.1[a]	25.6
Montmorency	0.3 ± 0.2[ab]	0.3 ± 0.2[ab]	3.5 ± 1.0[b]	0.5 ± 0.2[b]	0.9 ± 0.3[b]	5.5
inhibition, %	72.1	50.0	62.0	81.0	92.7	78.5
Balaton	0.4 ± 0.4[ab]	0.4 ± 0.2[a]	4.4 ± 0.1[b]	1.2 ± 0.6[ab]	1.6 ± 0.4[b]	8.0
inhibition, %	64.0	33.3	52.2	55.5	86.7	68.8

[a] Data represent the mean and standard deviation of five analyses per treatment. Values are expressed on a cooked ground beef bases. [b] Means in the same columns bearing different superscripts are significantly different ($p < 0.05$).

strated a significant correlation ($r = 0.88$) between TBARS values and total COPs concentrations in cooked pork stored at 4 °C over 4 days.

HAAs. In the preliminary study, ground beef patties were fried at 170 °C (surface temperature) for 8 min per side for a total of 16 min, with a final internal temperature of 62 ± 3 °C. Analysis of samples revealed little or no HAA formation in the control patties or in those containing the cherry tissue. Subsequently, the cooking protocol used by Balogh et al. (1995), that is, 10 min per side (total of 20 min) at 225 °C (final internal temperature of 68 ± 3 °C), was used to generate sufficient HAAs to evaluate the inhibitory effect of cherry tissue on HAA formation.

The average recoveries of HAAs added to cooked ground beef patties, with or without added tart cherry tissue, were 75.3 ± 21, 74.9 ± 12.5, 83.8 ± 10.5, 72.7 ± 28, and 65.0 ± 10.5% for IQ, MeIQ, MeIQx, Di MeIQx, and PhIP, respectively. These recoveries are comparable to those reported by Knize et al. (1994) and Balogh et al. (1995). Knize et al. (1997) reported recoveries ranging from 35 to 98% for IQ, MeIQx, and DiMeIQx and from 9 to 63% for PhIP. The large standard error in the present study is also comparable to those reported by Knize et al. (1997) for quality control samples analyzed periodically over 2 years, for which relative standard deviations ranging from 22 to 38% were obtained.

Five HAAs, IQ, MeIQ, MeIQx, DiMeIQx, and PhIP, were consistently detected in both the control and cherry patties after cooking (Table 5), although it is recognized that more HAAs may be present in cooked meat (Knize et al., 1997). Analysis of variance revealed that the addition of cherry tissue significantly ($p < 0.05$) reduced the formation of these compounds. Statistical analysis also revealed no significant ($p < 0.05$) difference between Montmorency and Balaton cherry tissue as inhibitors of HAA formation during cooking. Adding tart cherry tissue to ground beef at the 11.5% level reduced the total detectable HAA concentrations in the cooked patties from 25.6 ng/g of meat (control patties) to 5.5 and 8.0 ng/g of meat (Montmorency and Balaton patties).

As expected, the dominant HAA present was PhIP, followed by MeIQx, with lesser amounts of DiMeIQx, MeIQx, and IQ. These results are consistent with those of Gross and Grüter (1992) and Knize et al. (1997). In the control patties, the concentrations of the individual HAAs were generally similar to those reported in beef patties fried at 230 °C for 10 min per side (Knize et al., 1994). Four HAAs (PhIP, MeIQx, DiMeIQx, and IQ) appeared to be responsible for the majority of mutagenic activity in beef patties cooked between 150 and 230 °C. Total quantities measured ranged from undetectable amounts (<0.1 ng/g) for some cooked patties to 40 ng/g in beef patties fried at 230 °C for 10 min per side (Knize et al., 1994).

The calculated inhibitory effect of tart cherry tissue on HAA formation is summarized in Table 5). Cherry tissue inhibited PhIP formation by 93% (Montmorency) and by 87% (Balaton). The inhibitory effect of cherry tissue was as good or better than that achieved by other compounds. Balogh et al. (1995) reported similar reductions for vitamin E either when added directly to ground beef patties before frying or by its application to the surface of the patties before frying. Several studies have suggested other additives as inhibitors of mutagenic activity: Jones and Weisburger (1988) used a cooking sauce containing L-tryptophan to inhibit mutagenicity in fried or broiled ground beef patties. Skog et al. (1992) reduced mutagenic activity in fried ground beef patties by mixing glucose, lactose, powdered milk, or a potato starch/glucose combination with the meat before frying. Weisburger et al. (1994) reported that black tea, green tea, and the corresponding polyphenols could appreciably lower the formation of HAAs in a model system.

Significant reductions in PhIP concentrations (92−99%) in whole chicken breasts were achieved through marination with a mixture of brown sugar, olive oil, cider vinegar, garlic, mustard, lemon juice, and salt before grilling for 10−40 min (Salmon et al., 1997). Conversely, MeIQx concentrations increased >10-fold with marination, but only at the 30 and 40 min cooking times. Mutagenic activity was similarly lower in marinated samples cooked for 10, 20, and 30 min but higher in the marinated samples cooked for 40 min when compared with the marinated control. No mutagenicity studies were carried out with the cooked cherry patties. These will be done in further investigations, although it is expected that mutagenicity will decrease as all HAAs investigated were reduced by the inclusion of cherry tissue in the patties.

The reduction in HAA concentrations in the cherries patties was due in part to dilution as the patties contained 11.5% cherry tissue. However, the major inhibitory effect of cherry tissue is clearly due to cherry components functioning as inhibitors of the reaction(s) leading to HAA formation. It remains to be established which cherry compounds contribute this inhibitory effect, although it is anticipated that compounds with antioxidant activity are involved (Balogh et al., 1995).

The mechanisms by which antioxidants inhibit mutagen formation have not been fully elucidated. One possible mechanism is that phenolic antioxidants function as free radical scavengers and act in the early stages of the Maillard reaction prior to the Amadori rearrangement. Nyhammar (1986) proposed that pyrazine and pyridine radicals are intermediates in the formation of HAAs, whereas Namiki and Hayashi (1981) demonstrated the formation of free radicals, probably *N*,*N*′-disubstituted pyrazine cation radical products, through sugar fragmentation in the early stages of the Maillard reaction. Pearson et al. (1992) speculated that

the free radical scavenger-type antioxidants may stabilize the sugar fragment or else react with the free radicals formed by the Maillard reaction (either with alkylpyridine free radicals or with dialkylpyrazine free radicals). These theories are based on the premise that sugars are involved in HAA formation. However, it has been demonstrated that HAAs are formed in dry-heated mixtures of amino acids and creatine (Taylor et al., 1987). Clearly, there is a need to further study the mechanism of HAA formation in meats and how the reactions involved are influenced by phenolic compounds.

Conclusions. This study demonstrated that tart cherry tissue contains active antioxidant compounds that prevent the rapid formation of oxidation products in cooked ground beef patties during refrigerated storage. Tart cherry tissue also effectively reduced the formation of HAAs in cooked beef patties to levels normally formed in patties fried at lower temperature. More research is required to provide a better understanding of what compounds in cherry tissue are responsible for the inhibition of HAA formation and to establish whether the inhibiting effects of cherry tissue are dose-dependent.

ABBREVIATIONS USED

DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (A63100); IQ, 2-amino-3 methylimidazo[4,5-*f*]-quinoline (A61650); MeIQ, 2-amino-3,4-dimethylimidazo-[4,5-*f*]quinoline (A60520); MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (A60660); PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (A61700).

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