Antioxidative Characteristics of Oils in Ground Pork-Fat Patties Cooked with Soy Sauce

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ABSTRACT: Ground pork-fat patties were cooked alone (control), with NaCl solution, with NaCl solution containing sucrose, with fermented soybean broth, with fermented black soybean broth, and with commercial soy sauce products at 125°C for 2 h. The top-layer oils were then stored at 60°C for periodical determinations of conjugated diene hydroperoxide (CDHP) contents. The fermented broth-cooked and soy sauce-cooked oils were stable against CDHP formation during storage. This supports the customary impression that soy sauce-cooked pork is stable against oxidative rancidity. Analysis of the fatty acid compositions showed the presence of linoleic, linolenic, and arachidonic acids, which are susceptible to oxidation. When the fermented broth-cooked oils were extracted with methanol, concentrated, and introduced to the control oils for storage, an estimated antioxidative potency close to that of 2 ppm butylated hydroxyanisole was observed. In comparing free amino acid compositions of the fermented soybean and black soybean broths before and after cooking the ground pork-fat patties, threonine, serine, glutamic acid, alanine, isoleucine, leucine, tryptophan, and tyrosine contents decreased while proline, valine, histidine, and arginine contents increased.

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In Asian cuisine, pork loaves or slices cooked with soy sauce for a prolonged time are commonly used to prepare unique pork dishes. Formerly, when refrigeration was not widely used, soy sauce-cooked porks were stored at ambient temperature and reheated for service. It was observed then that oxidative rancidity of the soy sauce-cooked pork was unusual. However, for other types of pork, such as cooked meats, frozen pork sausage, cooked ground-pork patties and salted ground pork, oxidative rancidity or formation of warmed-over flavor in the products led to extensive concerns and investigations (1–3).

Hodge and Rist (4) first reported the lipid antioxidant effects of Maillard reaction products (MRP) for preserving vegetable oils. Lingnert and Lundgren (5) examined the antioxidative activity of MRP and tried to identify the compounds as food antioxidants to be used in place of synthetic antioxidants. Various MRP have been added to frozen pork sausage, cooked ground pork patties, and cooked ground beef for inhibiting oxidative rancidity (1,2,5–7). Soy sauce is rich in amino acids, peptides, and simple sugars, providing abundant precursors for Maillard reaction (8–10). Thus, the MRP formed in soy sauce-cooked products could retard oxidative rancidity.

This study is based on the fact that the antioxidative property in soy sauce-cooked pork is evident even though related scientific studies are meager. Various commercial soy sauce products were collected to cook ground pork-fat patties for characterization of the resultant oils. The investigations were extended to use fermented soybean and black soybean broths, prepared in our laboratory, to cook ground pork-fat patties for further characterization. Methanol extraction of antioxidants from the cooked oils and further incorporation of the concentrates to control oils for inhibiting oxidative rancidity were conducted. Changes in the fatty acid composition of the oils after a 10-d storage at 60°C, and free amino acid compositions of the fermented soybean and black soybean broth before and after cooking with the ground pork-fat patties, were also analyzed.

MATERIALS AND METHODS

Commercial soy sauce and fermented soybean and black soybean broth. Ten different commercial soy sauce products were collected from local supermarkets. Fermented soybean and fermented black soybean broths were prepared in our laboratory. Salinity of the commercial soy sauce products and of both fermented soybean and black soybean broths were around 15%, determined by volumetric titration with $AgNO_3$ using the Mohr method (11).

For preparation of the fermented soybean broth, soybeans were soaked in tap water for 6 h, steam-cooked for 40 min, spread onto a bamboo tray and cooled to *ca.* 40°C. Meanwhile, an equal amount of wheat grain was stir-fried until a light brown color was achieved. The grains were cooled, ground into coarse meal with a coffee mill, and mixed with 0.1% (in proportion to the original weight of dry soybean and wheat grain) of a commercial conidial powder (inoculum *ca.* 10^7 CFU/g) of *Aspergillus oryzae*. Then, the cooked soybeans were introduced, mixed, spread onto a bamboo tray, covered with two layers of cheesecloth and incubated at room temperature

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(24–29°C) for koji preparation (12). After incubation for 96 h, the mold-grown koji was weighed, deposited into a glass jar, and filled with 1.4 times (vol/wt) of a brine solution (20% NaCl, wt/vol) for fermentation at ambient temperature (18–30°C) with occasional agitation. After 6 mon of fermentation, the fermented soybean broth was collected. For fermentation of a black soybean broth, the procedure of Chen and Chiou (13) for Inyu preparation was followed. The fermented black soybean broth was also collected after 6 mon of fermentation. Both fermented broths were stored at 4°C for use.

Ground pork-fat patties and cooking. Fresh pork fat (leaf fat) was obtained from a local butcher and finely ground into patties with a meat grinder. Heat treatment and formulation of the ground pork-fat patties to be cooked with soy sauce were based on a household procedure for preparation of soy sauce-cooked pork. For each treatment, a 60-g ground pork-fat patty was placed in a porcelain bowl. Except for the control, the ground pork-fat patties were placed in a series of bowls and mixed, respectively with 40 mL 7.5% NaCl solution, 40 mL 7.5% NaCl solution containing 6 g sucrose, 40 mL twofold-diluted (1:1 with water) fermented soybean or black soybean broth, and the 10 commercial soy sauce products diluted 1:1 with water. A bowl containing 60 g of the ground pork-fat patties cooked alone was a control. As a preliminary experiment, the contents of a series of bowls containing 60 g ground pork-fat patties and 40 mL two-fold-diluted fermented soybean or black bean broth were cooked in a forced-air oven at 125°C for 1, 2, 3, and 4 h, respectively. Potent antioxidative activity observed in the 2-h-cooked oils was not enhanced by further cooking. In addition, when a series of 60-g ground pork-fat patties were cooked alone for 1 to 4 h, all resultant oils were unstable during storage. Thus, a cooking time of 2 h was used in this study. Contents of the bowls during cooking were stirred with a pair of chopsticks every 0.5 h. After cooking and cooling to ambient temperature, aliquots (1.5 mL) of the top-layer oils were withdrawn and deposited into 1.5 mL-microfuge tubes for centrifuging (8,000 × *g* for 1 min). The supernatant oils were used in the following experiments.

Oil storage and determination of the conjugated diene hydroperoxide (CDHP) contents. For determination of CDHP contents in the oils during storage, the procedure of Chen and Chiou (14) was followed with minor modification. One milliliter aliquots of oil were deposited into a series of 20-mL brown vials and placed in an oven set at 60 ± 1°C. During storage, 2.5 μL of the oil was withdrawn and dissolved in 2.5 mL isooctane, and the optical density at 234 nm was measured. The sampling times included 0, 1, 3, 6, and 10 d. The CDHP content was expressed as the optical density (OD) units at 234 nm of a 0.1% (vol/vol) oil solution in isooctane.

Analysis of fatty acid composition. Oil samples before and after a 10-d storage at 60°C were subjected to analysis of fatty acid composition following the procedure of Chiou *et al.* (15). Each fatty acid content was expressed as the percentage of the chromatographic area in proportion to the total integrated area.

Analysis of amino acid composition of the fermented soybean and black soybean broth. For preparation of a sample, 60 g of the ground pork-fat patties was cooked with 40 mL twofold-diluted fermented soybean or black soybean broth at 125°C for 2 h. After cooking, the contents were transferred to a graduated cylinder and replenished with deionized water to make the bottom-layer volume (aqueous phase) up to 40 mL. After thorough mixing, 10-μL aliquots of the aqueous solution were withdrawn and mixed with 990 μL sample buffer (citrate buffer, pH 2.2; Pickering Laboratories, Inc., Mountain View, CA) in test tubes (1.3 × 10 cm); the tubes were capped with aluminum foil and heated in a boiling water bath for 10 min. Then the solution was membrane-filtered (0.2 μM) (polytetrafluoroethylene syringe filter; Nalge Co., Rochester, NY) and subjected to analysis using an amino acid analyzer (AAA LC 3000; Biotronik, Posttach, Germany). A standard amino acid solution (Sigma Chemical Co., St. Louis, MO) was run concurrently for estimation of each amino acid concentration. For analyzing the free amino acid composition in the broth prior to cooking, 10-μL aliquots of twofold-diluted fermented soybean or black soybean broth were mixed with 990 μL sample buffer (citrate buffer, pH 2.2) and subjected to amino acid analysis using the same procedure. Each amino acid was expressed as mg/mL of broth. Two replicate trials from each treatment were conducted.

Antioxidant extraction from the fermented broth-cooked oils. For preparation of an extraction sample, 180 g of fresh ground pork-fat patties were mixed with 120 mL of twofold-diluted fermented soybean or black soybean broth in a square porcelain tray (5 × 20 × 20 cm) and cooked in a forced-air oven at 125°C for 2 h. After cooling, the top-layer oil was collected and centrifuged (8,000 × *g* for 1 min). Then, 50 mL of the obtained oil was transferred into a 500-mL flask, mixed with 150 mL methanol, sealed with a silicone stopper, and vigorously shaken at 250 rpm for 1 h using a rotary shaker. The extraction was repeated three times, and the pooled methanol was concentrated to dryness using a rotary evaporator at 45°C. The residue was dissolved in 0.5 mL methanol and stored at 4°C for further investigation.

For investigation of antioxidant potency in the extracted concentrates, aliquots (1 mL) of the control oils in a series of 20-mL brown glass vials were supplemented with 50 μL of the concentrates. Meanwhile, 50 μL of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) solutions in ethanol with various concentrations were introduced in place of the concentrates to yield oils containing 0, 10, and 30 ppm of BHT or BHA. The vials were stored in an oven at 60°C for 15 d, and CDHP contents in the oils were determined periodically following the procedure described above.

At least duplicate experiments were conducted. Means of the determinations with standard deviation were reported.

RESULTS AND DISCUSSION

Increases of CDHP content of the soy sauce-cooked oils. Increases of CDHP content in the oils separated from ground pork-fat patties cooked alone (control), with NaCl solution, NaCl solution containing sucrose, various commercial soy

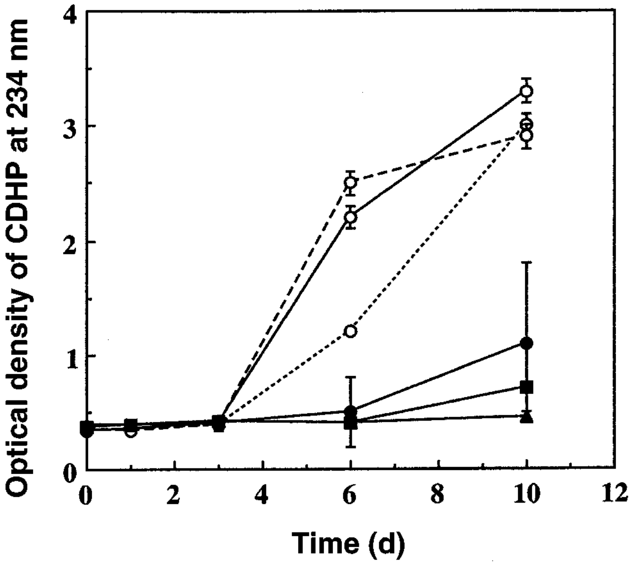

FIG. 1. Changes of conjugated diene hydroperoxide (CDHP) contents during storage at 60°C for 10 d of the oils separated from 60-g ground pork-fat patties after cooking. (—○—): cooked alone (control); (---○---): cooked with 40 mL 7.5% NaCl solution; (···○···): cooked with 40 mL 7.5% NaCl solution containing 6 g sucrose; (—●—): cooked with various twofold-diluted commercial soy sauce products; (—▲—): cooked with fermented soybean broth; (—■—): cooked with fermented black soybean broth. Error bars represent ± 1 SD.

sauce products, fermented soybean or black soybean broth, and subjected to storage at 60°C are shown in Figure 1. CDHP contents in the control oils and oils separated from ground pork-fat patties cooked with NaCl solution or NaCl solution containing sucrose increased significantly after 6 d of storage. This was in agreement with the report of Andersen and Skibsted (16) that the presence of NaCl enhances oxidative rancidity of frozen pork patties. Among the oils prepared from the ground pork-fat patties cooked with 10 commercial soy sauce products, CDHP content increased with time of storage and varied depending upon the nature of the individual product. When those oils were compared with the control oils and oils separated from ground pork-fat patties cooked with NaCl solution and/or the NaCl solution containing sucrose, CDHP contents increased much slower in the soy sauce-cooked oils than in the latter oils. This was supportive to the customary impression that soy sauce-cooked porks are stable against oxidation.

Soy sauce-involved browning reactions. When ground pork-fat patties were cooked with fermented soybean or black soybean broth, CDHP contents in the resultant oils did not increase during a 10-d storage at 60°C. Apparently antioxidative activity commenced in the pork fats after they were cooked with the commercial soy sauce products or with the fermented soybean and black soybean broth. Since fermented soybean or black soybean broth is the base of soy sauce (Shoyu) or black soy sauce (Inyu) formulation, the antioxidant precursors were mostly present in the fermented soybean and black soybean broth. In a preliminary test in which a series of ground pork-fat patties were cooked with fermented soybean or black soybean broth for various intervals, antioxidative potency in the 2-h-cooked oils was higher than in the 1-h-cooked oils and leveled off afterward (data not shown). This indicated that appropriate cooking was essential in generation of the antioxidative potency. During sauce fermentation, proteins and starches are extensively hydrolyzed to release amino acids, short-chain peptides, and reducing sugars in the resulting fermented broth, providing abundant precursors for the Maillard reaction (8–10,17). MRP were eventually formed through the Maillard reaction during cooking and rendering the products stable against oxidation. In addition to MRP, some natural antioxidants are present in ground pork (3), soybeans (18), and soybeans fermented with *A. saitoi* (19). Therefore, some natural antioxidants and/or fungal metabolites might also contribute to part of the antioxidative activity in the oils separated from ground pork-fat patties after cooking with soy sauce or fermented broth.

TABLE 1
Fatty Acid Composition of the Oils Separated from Ground Pork-Fat After Various Heat Treatments[a]

Fatty acid	Control	NaCl solution	NaCl solution and sucrose	Soybean broth	Black soybean broth
		Fatty acid content (% of total chromatographic area)[b]			
14:0	1.26 (1.44)	1.17 (1.42)	1.16 (1.39)	1.15 (1.22)	1.23 (1.22)
16:0	23.81 (27.31)	22.92 (26.88)	22.74 (26.77)	22.89 (23.74)	23.53 (23.31)
16:1	1.61 (1.67)	1.56 (1.61)	1.56 (1.65)	1.52 (1.52)	1.57 (1.61)
17:0	0.34 (0.36)	0.29 (0.31)	0.28 (0.40)	0.29 (0.28)	0.28 (0.29)
17:1	0.22 (0.25)	0.23 (0.26)	0.22 (0.24)	0.23 (0.23)	0.21 (0.23)
18:0	12.72 (14.93)	13.00 (14.93)	12.31 (14.71)	13.25 (13.48)	13.07 (12.84)
18:1	39.02 (42.20)	40.05 (42.63)	39.64 (42.42)	39.83 (39.88)	39.75 (39.70)
18:2	16.71 (8.66)	16.43 (8.40)	16.73 (8.42)	16.60 (15.04)	16.38 (16.54)
18:3	1.02 (0.26)	1.00 (0.25)	1.23 (0.25)	1.05 (0.91)	1.00 (1.02)
20:1	0.96 (1.01)	1.20 (1.05)	0.94 (1.03)	1.07 (1.05)	0.99 (0.99)
20:4	0.86 (0.48)	1.05 (0.57)	0.80 (0.40)	0.89 (0.77)	0.87 (0.91)
Total	98.53 (98.57)	98.90 (98.31)	97.61 (97.68)	98.77 (98.12)	98.88 (98.66)

[a]Fat cooked alone (control), with NaCl solution, with NaCl and sucrose, with soybean broth or with blackbean broth; all samples cooked at 125°C for 2 h and subjected to storage at 60°C for 10 d.
[b]Mean of determinations of replicate experiments ($n = 2$). Each data pair represents, respectively, the fatty acid content of each oil sample before and after storage.

TABLE 2
Free Amino Acid Compositions of Soybean and Black Soybean Fermented Broth Before and After Cooking Ground Pork-Fat Patties in These Broths at 125°C for 2 h

Amino acids[a] (mg/ml)	Soybean broth		Black soybean broth	
	Before cooking	After cooking	Before cooking	After cooking
Aspartic acid	5.28 ± 0.14	4.64 ± 0.32	7.80 ± 0.22	7.30 ± 0.12
Threonine	3.32 ± 0.26	1.70 ± 0.13	3.98 ± 0.16	2.10 ± 0.06
Serine	4.45 ± 0.39	3.08 ± 0.26	3.97 ± 0.19	2.38 ± 0.05
Glutamic acid	8.15 ± 0.80	4.90 ± 0.55	8.39 ± 0.29	6.06 ± 0.09
Proline	5.89 ± 0.73	6.98 ± 0.76	2.76 ± 0.01	4.72 ± 0.09
Glycine	1.93 ± 0.15	1.72 ± 0.13	1.54 ± 0.02	1.58 ± 0.07
Alanine	5.20 ± 0.25	2.22 ± 0.10	6.35 ± 0.63	2.38 ± 0.02
Valine	5.08 ± 0.11	5.46 ± 0.26	5.97 ± 0.25	7.54 ± 0.43
Methionine	1.30 ± 0.08	1.20 ± 0.11	2.43 ± 0.28	2.40 ± 0.17
Isoleucine	4.24 ± 0.25	3.00 ± 0.11	5.36 ± 0.33	4.06 ± 0.16
Leucine	5.95 ± 0.50	4.52 ± 0.23	6.25 ± 0.21	5.36 ± 0.12
Tyrosine	2.60 ± 0.27	1.22 ± 0.20	2.02 ± 0.23	1.80 ± 0.12
Phenylalanine	3.41 ± 0.02	3.00 ± 0.12	4.39 ± 0.10	2.94 ± 0.11
Lysine	1.67 ± 0.14	1.30 ± 0.07	2.44 ± 0.39	2.22 ± 0.27
Ammonia	1.05 ± 0.01	1.16 ± 0.01	1.15 ± 0.03	1.68 ± 0.02
Histidine	8.21 ± 1.20	14.98 ± 0.16	12.84 ± 0.52	18.96 ± 0.09
Tryptophan	8.89 ± 0.26	3.96 ± 0.01	7.99 ± 0.04	6.72 ± 0.40
Arginine	5.82 ± 0.08	12.12 ± 0.01	6.92 ± 0.49	13.23 ± 0.43
Total	86.06	77.16	92.55	93.43

[a]Mean ± SD of two determinations of duplicate experiments.

Changes of fatty acid composition. Fatty acid compositions of the oils separated from the ground pork-fat patties cooked alone, with NaCl solution, NaCl solution containing sucrose, fermented soybean or black soybean broth and subjected to oven storage at 60°C for 10 d are shown in Table 1. Prior to storage, fatty acid compositions of the oils prepared from different treatments varied slightly. However, after 10 d of storage, the compositions of control oils and oils separated from ground pork-fat patties cooked with NaCl solution and/or containing sucrose changed. Among the constituent fatty acids, linoleic acid (18:2), linolenic acid (18:3), and arachidonic acid (20:4) contents decreased after storage. In the oils separated from ground pork-fat patties cooked with the fermented soybean or black soybean broth, the fatty acid compositions did not change after storage. This was in agreement with the trend of CDHP changes in the oils subjected to storage (Fig. 1). This observation further confirmed the presence of antioxidants formed in the oils separated from ground pork-fat patties cooked with the fermented soybean or black soybean broth.

Changes of free amino acid composition. Free amino acid compositions of the fermented soybean and black soybean broth before and after cooking with ground pork-fat patties at 125°C for 2 h are shown in Table 2. Before cooking, the free amino acid profiles of fermented soybean and fermented black soybean broth varied over a limited range. In general, the contents of most amino acid decreased after cooking. The contents of threonine, serine, glutamic acid, alanine, isoleucine, leucine, tryptophan, and tyrosine decreased more pronouncedly than did those of other amino acids. However, proline, valine, histidine, and arginine contents increased after cooking. The latter amino acids probably were released from the ground pork-fat patties or from peptides in the fermented broth after cooking. The contents of some amino acids might decrease through chemical reactions, whereas some might increase through decomposition of macromolecules, so the overall changes in total free amino acid contents were not able to show the real chemical reactions. Nevertheless, there is no doubt that Maillard reactions took place intensively under the cooking condition and produced abundant MRP.

Antioxidative extracts and characterization. CDHP changes during storage of the control oils supplemented with the antioxidative extracts from ground pork-fat patties after cooking with fermented soybean or black soybean broth at 125°C for 2 h and supplementing with BHA and BHT are shown in Figure 2. Except for the control, CDHP contents in all oil samples did not increase up to 10 d of storage. After 15 d of storage, CDHP contents in the control oils supplemented with soybean broth-cooked extract, black soybean broth-cooked extract, and 10 ppm of BHT increased. In comparison, BHA was more effective than BHT in inhibiting CDHP formation. Based on the fact that changes of CDHP content in the control oils supplemented with both methanol extracts were close to that supplemented with 10 ppm BHT, the estimated antioxidative potency in the methanol extract-supplemented oils was equivalent to 10 ppm of BHT. Since the antioxidant concentration in the methanol extract-supplemented oils was five times greater than that in the original cooked-oils (1 mL of control oil was supplemented with 50 μL of methanol extract, in total 0.5 mL, which was extracted and concentrated from 50 mL of the original broth-cooked oil), the estimated antioxidative potency in the broth-cooked oils was close to that of 2 ppm BHT. This observation demonstrated that the antioxidants, mostly MRP, were methanol-extractable and that the potent antioxidative activity might be applicable for prevention of oxidative rancidity in

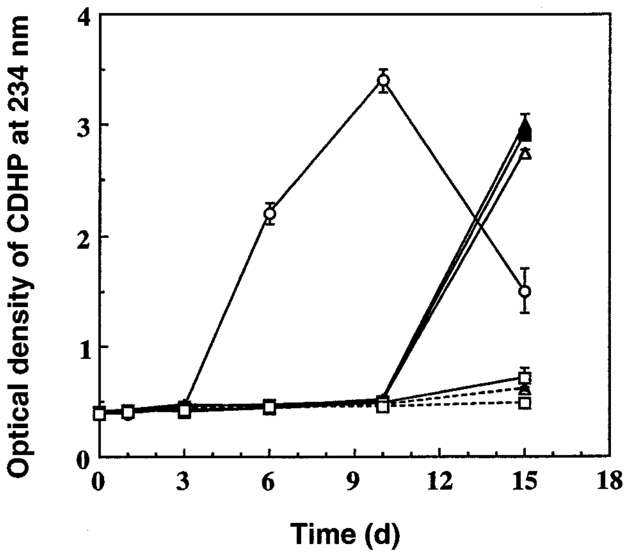

FIG. 2. Changes of CDHP contents during storage at 60°C for 15 d of the control oils supplemented with methanol extracts of the oils separated from ground pork-fat patties cooked with fermented soybean and black soybean broth and supplemented with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). (—○—): control; (—▲—): extract of soybean broth-cooked oils; (—■—): extract of black soybean broth-cooked oils; (—□—): 10 ppm BHA; (---□---): 30 ppm BHA; (—△—): 10 ppm BHT; (---△---): 30 ppm BHT. Error bars represent ± 1 SD. For other abbreviation see Figure 1.

foods. In the literature, MRP from glucose and histidine were observed to be effective in inhibiting rancidity development in frozen pork sausage (5,20). Bedinghaus and Ockerman (2) also applied the MRP from reducing sugars and free amino acids in cooked ground pork patties for antioxidation.

In conclusion, both commercial soy sauce products and fermented soybean or black soybean broth were potent in formation of MRP-antioxidants when combined with the ground pork-fat patties for cooking. The MRP-antioxidants in the oils were methanol-extractable and effective in inhibiting oxidative rancidity of the control oils. The antioxidative potency in the broth-cooked oils was close to that of 2 ppm BHT. Further investigations would be addressed on identification and characterization of the antioxidants and exploration of the applicable uses.

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