JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Bioactive Components of Caper (*Capparis spinosa* L.) from Sicily and Antioxidant Effects in a Red Meat Simulated Gastric Digestion

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An increasing body of evidence on the association between adherence to the Mediterranean diet and healthy status is being accumulated. Floral buds of *Capparis spinosa* L. are commonly used in the Mediterranean cuisine as flavoring for meat and other foods. The present study evaluated bioactive components and antioxidant activity of Sicilian capers stabilized in salt. Whereas α-tocopherol was absent, low levels of γ-tocopherol and vitamin C were measured. With reference to one serving size (8.6 g of capers), rutin was 13.76 mg, isothiocyanates, recently acknowledged as anticarcinogen phytochemicals, were 42.14 μmol, total phenols were 4.19 mg of gallic acid equivalents (GAE), and the total antioxidant potential measured using the [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS) cation radical decolorization assay was 25.8 μmol of Trolox equivalents. The antioxidative activity of a caper hydrophilic extract was assessed in a number of assays. The extract at 3.5 and 7.0 μM GAE exhibited a dose-dependent peroxyl radical scavenging activity in a methyl linoleate methanol solution oxidized by azo initiator, and reduced hypervalent iron myoglobin species formed from met-Mb an H_2O_2, at 180 μM GAE. The hydrophilic extract, at 70–280 μM GAE, caused a dose-dependent inhibition of lipid autoxidation in heated red meat, incubated with simulated gastric fluid for 180 min. In the same model rutin tested at a concentration corresponding to its content in the extract was ineffective, and α-tocopherol at 25 μM was poorly effective. The hydrophilic extract (70 μM GAE) prevented the consumption of the co-incubated α-tocopherol, whereas lipid oxidation was inhibited for the experimental time, suggesting cooperative interactions between extract components and the vitamin. The findings encourage the use of caper with foods that contribute oxidizable lipids in view of the association between dietary oxidized lipids and risk of oxidative stress-based diseases.

KEYWORDS: ***Capparis spinosa*; bioactive compounds; phytochemicals; isothiocyanates; rutin; dietary lipids; gastric fluid; myoglobin**

INTRODUCTION

Capparis spinosa L. (family Capparidaceae) is one of the most common aromatic plants growing wild in the dry regions around the Mediterranean basin. In Italy, this blue-gray spiny plant is intensively cultivated, particularly on small islands around Sicily, such as Pantelleria and the Eolian Salina, which provide 95% of the national product.

Capers are appreciated for their pungent and bitter flavor and are used as an appetizer with olives, cheese, and nuts or as a complement to meat, salads, pasta, and other foods. The aromatic floral buds are gathered just before blooming and stabilized either in brine or in salt. During this processing the plant glucosinolates are converted to their cognate isothiocyanates, responsible for the characteristic flavor of the caper, in a concomitant fermentation process (*1–3*). The fermentation is completed within 30 days, and edible capers are usually marketed after 2–3 months.

Apart from its use as flavoring, the caper has been known for centuries in traditional phytomedicine, which exploited its properties for several purposes. The aqueous extract from total aerial parts of the plant has been used for its antifungal (*4*), anti-inflammatory (*5*), antidiabetic (*6, 7*), and antihyperlipidemic (*8*) activities and is among the constituents of polyherbal formulations to treat liver ailments (*9*). Other investigations showed that raw floral buds contain lipids, alkaloids, glucocapperin as major glucosinolate, and a number of antioxidant phytochemicals such as flavonoids and other polyphenols (*2, 10–14*). Quercetin-3-rutinoside (rutin) has appeared to be common to most species and varieties, as well as the most abundant flavonoid in *C. spinosa* (*10–12, 14*). In addition, antioxidant properties of methanolic extracts of raw floral buds have been shown in various in vitro models (*12, 14*), and the potential

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10.1021/jf0714113 CCC: $37.00 © 2007 American Chemical Society
Published on Web 09/24/2007

use in oxidative stress-based pathological conditions has been suggested (*12, 15*). On the other hand, the antioxidative potential of the edible caper is less known. This study measured bioactive components and investigated the antioxidant potential of capers from Pantelleria (Sicily) in a number of assays, including measurement of total phenols, quenching of the $ABTS^{\bullet +}$, and reduction of peroxyl radicals and of hypervalent iron myoglobin species. In addition, considering the impact on human health of the postprandial oxidative stress associated with dietary oxidized lipids (*16–18*), antioxidant effects of caper were assessed in a heated meat autoxidation model mimicking the gastric digestion (*19*).

MATERIALS AND METHODS

Chemicals. Ammonium ferrous sulfate hexahydrate, ascorbic acid, 1,2-benzenedithiol, *trans*-β-carotene, gallic acid, hydrogen peroxide (H_2O_2), 13(*S*)-hydroperoxy-(9*Z*,11*E*)-octadienoic acid, horse skeletal muscle myoglobin (type I), linoleic acid methyl ester (LAME), phenyl isothiocyanate (phenyl-ITC), potassium persulfate ($K_2S_2O_8$), rutin, 2,6-di-*tert*-butyl-4-methylphenol (BHT), thiobarbituric acid (TBA), α-, β-, and γ-tocopherol, tricholoroacetic acid (TCA), and xylenol orange were from Sigma Chemical Co. (St. Louis, MO). [2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were from Aldrich Chemical Co. (Gillingham, U.K.). 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) was from Polyscience, Inc. (Warrington, PA). All other materials and solvents were of the highest purity or high-performance HPLC grade.

1,3-Benzodithiole-2-thione was synthesized by permitting phenyl-ITC to react with 1,2-benzenedithiol in a mixture of equal volumes of methanol and 100 mM potassium phosphate, pH 8.5, at 25 °C, and the product was crystallized from methylene dichloride according to Zhang's method (*20*). Spectrophotometric quantitation was at 365 nm using the extinction molar coefficient of 23000 M^{-1} cm^{-1} (*21*).

Simulated gastric fluid (SGF) was freshly prepared according to the Italian Pharmacopoeia (*22*). SGF contained 0.2% NaCl, 0.32% pepsin, and HCl to obtain pH 2.0.

Capers and Preparation of Caper Extracts. Capers (*C. spinosa* L.) from Pantelleria (Sicily) are fermented in salt. Raw capers, placed in cement basins, are treated with 40% sea salt (w/w) and stirred several times during 8 days. Then the capers are placed in other basins and treated again with 25% sea salt (w/w) for 15 days before packaging. In our study capers were obtained 12 months after packaging from the local "Cooperativa Agricola Produttori Capperi". Capers were washed three times with large amounts of 5 mM saline phosphate buffer, pH 7.4 (PBS), dried on blotting paper, and finely chopped. Samples (50 g) were then extracted with 100 mL of either methanol/water (2:1, v/v) or dichloromethane over 24 h at 4 °C. After a cleanup step via centrifugation and filtration through a Millex HV 0.45 μm filter (Millipore, Billerica, MA), the extracts were subjected to rotary evaporation to remove the organic solvents. Finally, the hydrophilic extract was submitted to cryodessication. All extracts were stored at −80 °C and analyzed within 2 months. Immediately before the use, the dried extracts were resuspended in the suitable medium at the required concentration.

HPLC Procedures. A Gilson modular liquid chromatographic system (Gilson Inc., Middleton, WI) equipped with M 302 and 305 pumps, an injector model 77-25 (Rheodyne, Berkeley, CA) with a 100 μL injector loop, and an M 802c manometric module was used. The chromatographic column was a 5 μm Chromsep C18 column (250 × 4.6 mm i.d.) with a 5 μm guard column (10 × 4 mm) (Varian, Palo Alto, CA). Detection was by an M 118 UV–visible detector used along with the Gilson 712 HPLC System Controller software. Sensitivity was 0.05% AUFS.

Tocopherols. Lipophilic extract relevant to 100–250 mg of caper was mixed with 1 volume of water and 2 volumes of absolute ethanol, followed by extraction with 8 volumes of petroleum ether. The extracts were gathered, dried under nitrogen, resuspended in several microliters of methanol, and injected on top of the HPLC column eluted with methanol at 1.5 mL min^{-1}. Spectrophotometric revelation was at 290 nm.

Carotenoids. Lipophilic extract relevant to 100–250 mg of caper was mixed with 2 volumes of methanol, 2 volumes of water, and 6 volumes of hexane/diethyl ether (1:1, v/v). The organic phase was dried under nitrogen, resuspended in several microliters of a mixture of acetonitrile/methanol/tetrahydrofuran (58.5:35.0:6.5, v/v/v), and injected on top of the HPLC column eluted with the same solvent at 2.0 mL min^{-1}. Spectrophotometric revelation was at 450 nm.

Vitamin C. Evaluation of ascorbic acid in the hydrophilic extract relevant to 10–50 mg of caper was performed by reversed-phase HPLC, with spectrophotometric detection at 266 nm, as reported by Lazzarino et al. (*23*) with minor changes. These included length of the column (250 × 4.6 mm i.d.), particle size (5 μm), and isocratic elution with 10 mM KH_2PO_4 buffer, pH 7.0, containing 1% methanol and 10 mL/L tetrabutylammonium bromide, at 1.2 mL min^{-1}. The retention time of ascorbate was 11.90 min.

Rutin. Rutin in the hydrophilic extract relevant to 1–5 mg of caper was evaluated by reversed-phase HPLC as reported (*14*), using an isocratic elution with methanol/acetic acid 1% aqueous solution, 43:57 (v/v), at a flow rate of 1.0 mL/min. Revelation was at 350 nm.

Total ITCs. Total ITCs were determined using a cyclocondensation reaction with 1,2-benzenedithiol, which led to a single product, 1,3-benzodithiole-2-thione, detected on reversed-phase HPLC (*20*). In brief, hydrophilic extract relevant to 100–500 mg of caper, in 100 μL of methanol, was mixed with 600 μL of 1,2-benzenedithiol in 2-propanol (10 mM, degassed) and 500 μL of potassium phosphate (0.1 M, pH 8.5, degassed). After a 2 h incubation at 65 °C, samples were centrifuged at 3000*g* for 20 min. Aliquots of the supernatants were injected on top of the HPLC column eluted with a mixture of methanol/water (70:30, v:v) at 1.0 mL min^{-1}. Spectrophotometric revelation was at 365 nm.

All compounds were quantified by reference to standard curves constructed with 1–100 ng of each pure compound.

Total Antioxidant Potential. The total antioxidant activity (TAA) of both the hydrophilic and lipophilic extracts was evaluated using the ABTS radical cation decolorization assay (*24*). $ABTS^{\bullet +}$ was prepared by reacting ABTS with $K_2S_2O_4$ (*25*). Samples were analyzed in duplicate, at five different dilutions, within the linearity range of the assay. The assay was standardized with the synthetic antioxidant Trolox, and results were expressed as micromoles of Trolox equivalents per gram of fresh weight .

Total Phenols. The Folin–Ciocalteu method, based on the reduction of phosphotungstic–phosphomolybdic acid (Folin–Ciocalteu's reagent) to blue reaction products in alkaline solution, was used to determine phenolic compounds in hydrophilic extracts (*26*). Extracts relevant to 10–100 mg of caper were analyzed in duplicate. Quantitation was by reference to curves constructed with gallic acid, and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight.

Peroxyl Radical Scavenging Assay in Solution. Peroxidation of methyl linoleate was performed by incubating 300 mM LAME and 2.0 mM AMVN, in a final methanol volume of 1.0 mL, in a water bath at 37 °C, under air. Portions of the mixture (10 μL) were taken at intervals and injected onto a Supelco Supelcosil (Bellefonte, PA) LC-18 column (250 × 4.6 mm i.d., 5 μm), equilibrated, and then eluted with methanol at a flow rate of 1.0 mL/min. Quantitation was by reference to a standard curve constructed with known amounts of linoleic acid hydroperoxide. When required, suitable amounts of either vitamin E or hydrophilic caper extract in methanol were added to the solution of methyl linoleate and allowed to equilibrate at 37 °C for 60 s. The azoinitiator was added, and the incubation was carried out as above.

Hypervalent Iron Mb Species Reduction Assay. Oxidation of horse met-myoglobin (Mb) was carried out, at 37 °C, in a reaction mixture containing 50 μM hemoprotein in 0.1 M sodium acetate, pH 5.0, supplemented with 50 μM H_2O_2, either in the absence or in the presence of either hydrophilic caper extract resuspended in the same buffer or Trolox. Trolox was prepared as ethanol solution, and the final concentration of ethanol in the assay reaction mixture did not exceed

Table 1. Bioactive Compounds and Antioxidant Potential of Caper (*Capparis spinosa* L.) from Sicily

	amount[a]	*n*
lipophilic extract		
vitamin E (μg/100 g of capers)		
α-tocopherol	nd	3
β-tocopherol	nd	3
γ-tocopherol	0.8 ± 0.1	3
β-carotene	nd	2
hydrophilic extract		
vitamin C (mg/100 g of capers)	0.1 ± 0.02	4
rutin (g/100 g of capers)	0.16 ± 0.02	5
total ITCs (μmol/100 g of capers)	4.9 ± 0.5	5
total phenols[b]	48.75 ± 5.0	6
hydrophilic TAA[c]	2.37 ± 0.12	6
lipophilic TAA[c]	0.63 ± 0.05	5

[a] Each value is the mean ± SD of *n* determinations performed in duplicate. nd, not detectable. [b] Milligrams of GAE/100 g of capers. [c] Micromoles of Trolox equivalents/g of capers.

0.1%. Myoglobin spectral changes in the visible region were measured on a Beckman DU 640 UV–vis spectrophotometer, equipped with a temperature controller.

Lipid Peroxidation in Simulated Gastric Fluid. Grilled turkey red muscle, in the form of small slices, was divided into portions and kept at −80 °C until the experiment. The tissue was ground with 3 volumes of SGF (w/v) for 2 min in a laboratory blender (Waring, New Hartford, CT). When required, SGF contained caper hydrophilic extract and/or ethanolic vitamin E (0.1% final ethanol concentration) or rutin in DMSO (0.1% final DMSO concentration).

The homogenate was adjusted to pH 3.0 with HCl and incubated at 37 °C in a shaking bath for 180 min. Suitable aliquots were withdrawn at different time points, and lipid peroxidation was monitored by the formation of both hydroperoxides and TBA-reactive substance (TBA-RS). Hydroperoxides in the samples were extracted by mixing with 1 methanol volume. After centrifugation at 3000*g* for 15 min at 4 °C, aliquots (200 μL) of the supernatant were subjected to the FOX2 assay. The FOX2 test system is based on the oxidation of ferrous ion to ferric ion by hydroperoxides to produce a ferric oxidation–xylenol orange complex with absorbance peak at 560 nm (*27*) The FOX reagent contained 250 μM ammonium ferrous sulfate, 100 μM xylenol orange, 25 mM H_2SO_4, and 4 mM BHT in 90% (v/v) methanol. The blank reagent contained all components of the solution except ferrous sulfate. Hydroperoxides were evaluated as the absorbance difference between test and blank tubes, using a solution of H_2O_2 as a standard.

TBA-RS (*28*) were evaluated in aliquots (200 μL) of the reaction mixture, diluted with H_2O to a final volume of 1 mL, and added to 2 mL of a solution containing 15% TCA (v/v), 0.375% TBA (w/v), 0.25 N HCl, and 0.02% BHT (w/v). The mixture was incubated in a boiling water bath for 30 min. After cooling, the precipitate was removed by centrifugation, and TBA-RS in the supernatant were determined at 532 nm. The results were expressed as nanomoles per milliliter of MDA equivalents, using the molar extinction coefficient of 156000 (*28*).

Co-oxidation of vitamin E in the meat–SGF mixture was evaluated by extraction of the vitamin followed by HPLC analysis as above reported.

RESULTS

Table 1 shows the amount of antioxidant vitamins and peculiar phytochemicals in extracts of the commercial Sicilian caper preserved in salt. Very small amounts of vitamin E as γ-tocopherol were found in the lipophilic extract, and vitamin C in the hydrophilic extract appeared modest. Rutin accounted for 0.16% (w/w), and significant amounts of total ITCs were measured. **Table 1** also reports the antioxidant potential of the caper in terms of total phenols and total antioxidant activity, as measured by quenching of $ABTS^{\bullet+}$, in both the hydrophilic and the lipophilic extracts.

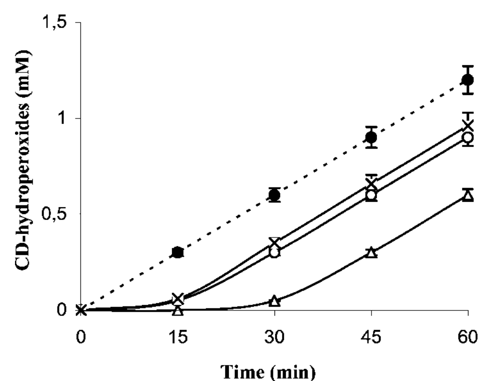

Figure 1. AMVN-induced oxidation of methyl linoleate in methanol in the absence (dotted line) or in the presence of hydrophilic extract from caper (3.5 μM GAE, ○; 7.0 μM GAE, △) or 5 μM vitamin E (×). Oxidation conditions are reported under Materials and Methods. Each point represents the mean ± SD of three determination carried out with different incubation mixtures.

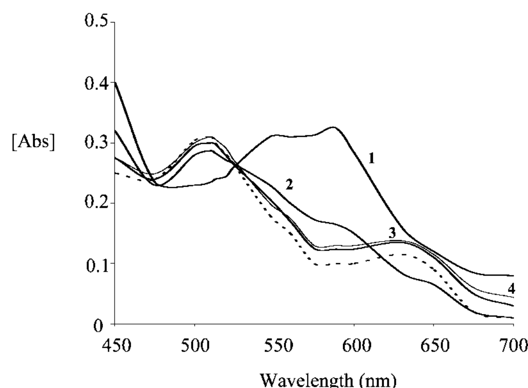

Figure 2. Spectral changes of ferryl-Mb upon reaction with hydrophilic extract from caper. Met-Mb (50 μM; dotted line), at pH 5.0, was converted to ferryl-Mb by a 1 min incubation with H_2O_2 (50 μM; line 1). Hydrophilic extract from caper (180 μM GAE) or Trolox (250 μM) was added to the incubation mixture before H_2O_2. Lines 2 and 3 are scans in the presence of caper extract at 2 and 8 min, respectively; line 4 is a scan in the presence of Trolox at 8 min.

The antioxidant activity of the hydrophilic extract from caper was checked in methyl linoleate methanol solutions oxidized by AMVN. A net dose-dependent delay of the hydroperoxide formation was observed in the presence of 3.5 and 7.0 μM GAE caper extract (**Figure 1**). The effect of 3.5 μM GAE caper extract was comparable with that of 5 μM vitamin E, with an inhibition period around 15 min (**Figure 1**).

Reducing activity of the caper extract toward ferryl-Mb, a highly reactive hypervalent iron myoglobin species, was investigated. Purified met-Mb was treated with H_2O_2 to generate ferryl-Mb, and the reaction was monitored by the spectral changes between 450 and 700 nm. A typical experiment is represented in **Figure 2**. When 50 μM met-Mb was incubated with a stoichiometric amount of H_2O_2, at 37 °C and pH 5.0 for 1 min, the characteristic peak of met-Mb at 630 nm (**Figure 2**, dotted line) was completely lost, whereas two new peaks at 545 and 580 nm, corresponding to the ferryl-Mb, appeared (**Figure 2**, line 1). A spontaneous autoreduction of ferryl-Mb to met-Mb was not spectrophotometrically evident within the following 10 min of observation (not shown). When caper extract (180 μM GAE) was included in the mixture before the addition of H_2O_2, a remarkably lower amount of ferryl-Mb was formed after 1 min of incubation (**Figure 2** line 2). In addition, spectral variations monitored at 1 min intervals showed a reduction of

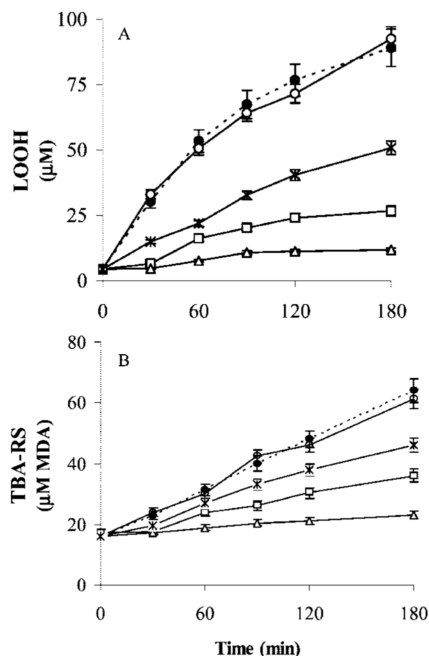

Figure 3. Time course of hydroperoxide (**A**) and TBA-RS formation (**B**) during the autoxidation of heated red meat in SGF, pH 3.0, in the absence (dotted line) or in the presence of hydrophilic extract from caper (70 μM GAE) (×), 140 μM GAE (□), 280 μM GAE (△), or 1.2 mM rutin (○). Incubation conditions and measurement of hydroperoxides and thiobarbituric acid reactive compounds as MDA are reported under Materials and Methods. Each value is the mean ± SD of four determinations performed in duplicate.

the ferryl form to the met-Mb species within 8 min (**Figure 2**, line 3). As a comparison Trolox, a water-soluble analogue of vitamin E known to reduce ferryl moieties (*29, 30*), brought about a similar reduction pattern when included in the incubation mixture at 250 μM (**Figure 2**).

Antioxidative effects of the hydrophilic extract were assessed during lipid autoxidation of heated red meat, in simulated gastric fluid, by monitoring the formation of lipid hydroperoxides and TBA-RS for 180 min (**Figure 3**). When corrected for the weight/volume dilution ratio with SGF, the basal level of oxidized lipid in grilled muscle accounted for 12 ± 0.9 μmol of hydroperoxides and 45 ± 3.2 μmol of TBA-RS (n = 4) per gram of grilled muscle. Increases of 6- and 3-fold of hydroperoxides and TBA-RS, respectively, were measured at the end of incubation. Lipid peroxidation was inhibited by the hydrophilic extract from caper (70–280 μM GAE), in a dose-dependent manner, whereas rutin at 1.2 mM appeared to be ineffective (**Figure 3**). Vitamin E is the main lipid antioxidant in biological environments. The antioxidant effect of α-tocopherol in the meat autoxidation process was evaluated, along with the time course of the vitamin consumption, and the results are shown in **Figure 4**. Vitamin E at 25 μM was poorly effective, whereas it was consumed during lipid oxidation (**Figure 4**). Co-incubation of caper extract (70 μM GAE) with vitamin E (25 μM) completely inhibited lipid oxidation for the experimental time, providing evidence of cooperative antioxidant effects, whereras the vitamin appeared to be preserved for 60 min (**Figure 4**). In additional experiments red meat was homogenized in SGF in the presence of whole caper and submitted to autoxidation. Amounts of 35 and 86 mg og caper per milliliter of homogenate (140 and 280 μM GAE) decreased the time-dependent formation of lipid hydroperoxides in a dose-dependent manner (**Figure 5**).

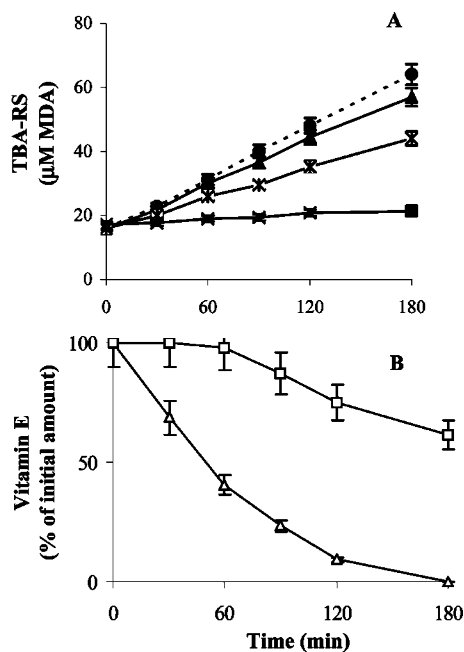

Figure 4. (**A**) TBA-RS formation during the autoxidation of heated red meat in SGF, pH 3.0, in the absence (dotted line) or in the presence of 25 μM vitamin E (▲), hydrophilic extract from caper (70 μM GAE) (×), and 25 μM vitamin E plus caper extract (70 μM GAE) (■). (**B**) Consumption of the antioxidant, at 25 μM, alone (△) or in combination with the caper extract (70 μM GAE) (□). Incubation conditions and measurement of thiobarbituric acid reactive compounds as MDA and vitamin E are reported under Materials and Methods. Each value is the mean ± SD of three determinations performed in duplicate.

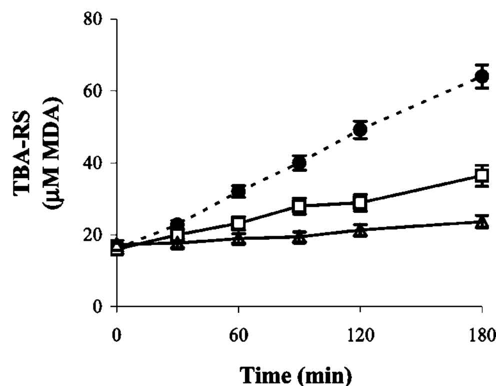

Figure 5. Effect of whole caper on the autoxidation of heated red meat: TBA-RS formation in the red meat homogenate in SGF, pH 3.0 (300 mg/mL), prepared in the absence (dotted line) or in the presence of either 35 mg (□) or 86 mg (△) of caper per milliliter of homogenate. Incubation conditions and measurements of TBA-RS as MDA are reported under Materials and Methods. Each value is the mean ± SD of three determinations performed in duplicate.

DISCUSSION

Assessing antioxidant vitamins and phytochemicals such as polyphenols, flavonoids, and other bioactive compounds is a tool to explore potential benefits of plant foods on human health, on the basis of the putative role of these components in the prevention of a number of degenerative and age-related disorders. This study shows that the edible caper, although poor in tocopherols and vitamin C, contains substantial amounts of ITC. The compounds that are formed from the parent glucosinolates by the activity of myrosinase, a vacuolar hydrolytic enzyme released following the salt processing (*1–3*), occur to an extent

comparable to that reported in several cruciferous vegetables, which are a main source of these compounds in the human diet (*32*). Dietary isothiocyanates are widely regarded as potentially important chemoprotectors against cancer (*32–35*). They are capable of interrupting several steps in the carcinogenic process, including protection of DNA by inhibition of carcinogen-activating enzymes (*36, 37*) and induction of carcinogen-detoxifying enzymes (*38, 39*). In vivo studies in humans showed that the bioavailability of isothiocyanates from broccoli sprouts is high (*40*). Although comparable studies have not been reported for caper yet, it is plausible to suggest that caper may be a dietary source of interest for this class of bioactive compounds.

Caper has appeared to be a relatively rich source of antioxidant phytochemicals such as flavonoids (*11, 12, 14*). The flavonoid profile has appeared common to a large number of *Capparis* species, with quercetin and kaempferol glycosides being the most represented, but the relative amounts of individual flavonoids has appeared quite different (*11*). In addition, whereas the salt processing maintains the glycosylated forms, brining causes a very extensive hydrolysis (*11*). This may be of interest in that aglycones are much more susceptible to oxidative degradation, which may affect the antioxidative potential of commercial caper. In our caper from Pantelleria, which was processed in salt, rutin was found to the extent of 1.6 mg/g, an amount that is about 50% that reported in raw capers of the same cultivar (*14*). This shows that the industrial salt processing causes some degradation, thus affecting the level of such a compound. In any instance, according to our measurements, one serving of caper [8.6 g (*41*)] provides an amount of rutin no less than that of a 100 g serving of fried onions (*42*), considered to be a good dietary source of such flavonoid.

When compared with the antioxidant capacity reported for a large number of vegetables in terms of TP and TAA (*43, 44*), the caper cannot be ranked among the dietary components with high antioxidant potential. Nevertheless, we showed that a caper extract as small as 70 μM GAE was effective in preventing lipid autoxidation of red meat during a simulated gastric digestion. The bioactive components of the caper extract per se cannot be easily reconciled with the observed effect. Although isothiocyanates are powerful electrophiles, there is no chemical or biochemical evidence that they can participate in oxidation or reduction reactions as direct-acting antioxidants (*45*). In addition, the ineffectiveness of rutin at inhibiting red meat autoxidation, when tested at concentrations corresponding to its content in the extract, would rule out a mere contribution of this component in our model. Other caper components such as phenolic acids (*12*) can be considered. In any instance, acting in synergism, antioxidant phytochemicals may provide an appropriate protection even when occurring in small amounts (*46, 47*). In addition, interactions with other food antioxidants may enhance the efficacy. We observed cooperative effects with vitamin E, with concurrent protection of the vitamin, suggesting the activity of caper components capable of recycling α-tocopherol. Regeneration of α-tocopherol by flavonols and their glycosides has been reported either in solution (*48*) or in red blood cells (*49*).

Meat components can be remarkably altered in the digestion process in the stomach, through the activity of endogenous catalysts such as met-Mb (*19, 50–52*). Lipid hydroperoxides from heated meat may stimulate the hydroperoxidase activity of met-Mb (*53*), resulting in the generation of a hypervalent iron ferryl species (*54*). The acidic pH of the gastric fluid stabilizes a highly instable protonated form of ferryl-Mb (*55*), which initiates lipid oxidation. Our findings that the caper extract was capable of reducing ferryl-Mb, as well as scavenging chain-carrying peroxyl radicals, suggest that the protective effect on the red meat autoxidation may involve inhibition of initiation as well as propagation steps of lipid oxidation. In contrast, possibly due to the presence of preformed hydroperoxides and pro-oxidant involvement, which affects the chain process of lipid oxidation, vitamin E cannot act as an effective chain-breaking antioxidant in the meat autoxidation model, although it is consumed.

Lipid oxidation during digestion co-oxidizes antioxidant vitamins (*19, 52*), thus affecting the nutritional value of various foods, and generates a number of cytotoxic and mutagenic compounds including hydroperoxides, malondialdehyde, and hydroxy alkanals (*56–58*). This contributes to a postprandial oxidative stress (*16–18*), a condition important in modulating cardiovascular risk (*18, 60, 61*). Because of their antioxidant components, fruits and vegetables are recommended in a worldwide campaign of disease prevention. By inhibiting the accumulation of harmful lipid oxidation products and increasing the level of bioavailable vitamin E, caper may have beneficial health effects, especially for people whose meals are rich in fats and red meats. It may be worthwhile to mention that the antioxidative activity was evident at a caper/red meat ratio of 1:10 (w/w), which is consistent with the use of the caper buds as a cooking flavoring.

ACKNOWLEDGMENT

We thank Rosario Cappadona from the Cooperativa Agricola Produttori Capperi, a.r.l., Pantelleria.

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Received for review May 14, 2007. Revised manuscript received July 26, 2007. Accepted August 2, 2007. This work has been carried out with a grant from Assessorato Agricoltura e Foreste Regione Sicilia.

JF0714113