JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Phenolic Composition and Antioxidant Activity of Aqueous Infusions from *Capparis spinosa* L. and *Crithmum maritimum* L. before and after Submission to a Two-Step in Vitro Digestion Model

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ABSTRACT: This study investigated the phenolic composition and antioxidant activities of aqueous infusions from wild-grown caper (*Capparis spinosa* L.) and sea fennel (*Crithmum maritimum* L.) from the Dalmatia region (Croatia) before and after their submission to an in vitro digestion process. HPLC/UV-vis-DAD/ESI-MS analysis of the caper infusion identified rutin, kaempferol 3-*O*-rutinoside, and isorhamnetin 3-*O*-rutinoside as dominant flavonoids in the matrix together with a series of cinnamoylquinic acid derivatives, whereas in the sea fennel aqueous infusion chlorogenic acid (5-caffeoylquinic acid), its isomers, and higher derivatives were identified as almost the sole class of phenolics. Both infusions exhibited good and dose-dependent antioxidant activity before in vitro digestion by the DPPH method, the β-carotene bleaching method, and copper-induced oxidation of human LDL. The amount of total phenolics (Folin−Ciocalteu assay) strongly decreased in digested samples (from 3.0 and 2.2% in caper and sea fennel infusions, respectively, to <1.0%), as did their antioxidant activity as measured by the three aforesaid methods. The results showed that the majority of phenolic compounds detected in both infusions are not stable under applied simulated gastrointestinal conditions and that the stability of these secondary metabolites strongly depends on the nature of the corresponding matrix.

KEYWORDS: caper, sea fennel, flavonoids, phenolic acids, antioxidant activity, in vitro digestion

INTRODUCTION

Caper (*Capparis spinosa* L.) and sea fennel (*Crithmum maritimum* L.) are aromatic plants present in the Mediterranean diet and rich in compounds with high antioxidant potential. Capers are flower buds of the *Capparis* genus, native to the Mediterranean region, growing wild on walls or in rocky coastal areas. Caper has been used in phytomedicine since ancient times as an antioxidant, antifungal, antihepatotoxic, anti-inflammatory, and antidiabetic.[1,2] Phytochemical studies on caper extracts reported the presence of alkaloids, fatty acids, phenolic acids, flavonoids, vitamins, and glucosinolates as the main chemical constituents.[1,3−7]

Sea fennel is an edible wild plant with characteristic yellow flowers, growing on maritime rocks along the Mediterranean, Pacific, and Atlantic coasts; it is used for culinary purposes as a pickle and for flavoring and sometimes as a substitute for caper. Although this plant has been investigated mainly for its essential oil content,[8−10] it is reported to be rich in several other nonvolatile compounds, such as ascorbic acid, carotenoids, flavonoids, and generally bioactive components, which could be used for aromatic, medicinal, antimicrobial, and insecticide purposes.[8,11−14] Both caper and sea fennel are very good sources of phenolic compounds, which are known to provide health-improving benefits due to their various biological activities (antioxidant, anticarcinogenic, antimicrobial, antimutagenic). The biological activity of phenolic compounds is generally suppressed by metabolism;[15] in fact, it was found that colonic microflora extensively converts most dietary phenols into different molecules,[16] thus modifying their chemical structures in a not easily predictable way.[1] Such changes are very important as they reflect changes in their biological properties (e.g., antioxidant activity), which are known to be structure-related. Given the knowledge of the effects of digestive pathways on biologically active compounds is still fragmentary, an in vitro model is very useful because it simulates the digestive processes in the gastrointestinal tract in a simplified manner, providing important information on the stability and putative modifications of compounds of interest under gastrointestinal conditions.

In our previous study, the chemical composition and antioxidant activity of the essential oils from wild-grown caper and sea fennel from Dalmatia (Croatia) were investigated.[10] The aim of the present paper was to extend the investigation to the phenolic composition and the antioxidant activity of aqueous infusions from the same plants and to explore if, and to what extent, simulated gastrointestinal conditions could affect both. Several studies on the stability of phenolic compounds submitted to in vitro digestion processes have been published recently;[17,18] this study also shows the influence of the original food matrix during such processes.

Received: August 2, 2011
Revised: October 20, 2011
Accepted: October 22, 2011
Published: October 22, 2011

dx.doi.org/10.1021/jf203096q | *J. Agric. Food Chem.* 2011, 59, 12453–12459

MATERIALS AND METHODS

Materials. Wild-grown caper (*Capparis spinosa* L.) and sea fennel (*Crithmum maritimum* L.) were collected in central Dalmatia (Croatia) in July. The voucher specimens (TKB 05-09 and TKB 03-01) are deposited in the Laboratory of Biochemistry, Faculty of Chemistry and Technology, Split, Croatia. For preparing aqueous infusions from caper and sea fennel, 15 g of the air-dried herb (flower tops and stalks) was infused into 150 mL of boiling distilled water for 30 min, filtered through Whatman no. 4 paper, and then concentrated under vacuum to dryness. The residue obtained was redissolved in water to a final concentration of 60 g/L. Determination of total phenolic content and antioxidant activity of original caper and sea fennel infusions was performed immediately after preparation procedure. Infusions were freeze-dried overnight at $T = -45$ °C and $P = 0.7-0.8$ mbar and then kept at −80 °C until analyses.

Chemicals. Unless otherwise stated, all solvents used in this study were high-purity spectroscopic grade solvents from Carlo Erba (Milano, Italy). Pure reference standards (kaempferol, kaempferol 3-*O*-glucoside, isorhamnetin, isorhamnetin-3-*O*-rutinoside, and chlorogenic acid) were purchased from Extrasynthese (Lyon, France); quercetin 3-*O*-glucoside, quercetin, and rutin were obtained from Fluka (Sigma-Aldrich S.r.l., Milano, Italy). 2,2′-Diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, Tween 40, β-carotene, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and digestion enzymes (pepsin and pancreatin) were purchased from Sigma-Aldrich (Switzerland). Folin−Ciocalteu reagent and gallic acid were purchased from Kemika (Croatia). The mixture of commercial, high-purity standards used in this study was prepared by dissolving 2.7 mg of chlorogenic acid, 1.8 mg of rutin, 1.2 mg of quercetin 3-*O*-glycoside, and 0.12 mg of quercetin in 5 mL of HPLC grade water; the solution was then brought to the final volume of 10 mL in an amber volumetric flask and stored at 4 °C until use. For the HPLC analyses, 250 μL of the standard solution was brought to 1 mL with HPLC grade water in a 2 mL vial and analyzed immediately.

Determination of the Total Phenolic Content. Undigested and digested aqueous infusion samples were evaluated according to the Folin−Ciocalteu colorimetric method,[19] expressing the results as gallic acid equivalents (GAE). All determinations were run in triplicate.

Measurement of Radical Scavenging Effect Using the DPPH Radical Scavenging Method. The radical scavenging activity of undigested and digested samples was evaluated as described in Kulisic et al.[20] Synthetic antioxidants (BHA and BHT, see the corresponding paragraph for description) were used as positive control, whereas working medium with ethanol was used as blank. All determinations were performed in triplicate.

Determination of Antioxidant Activity Using the β-Carotene Bleaching (BCB) Method. The antioxidant activity of tested samples, expressed through the inhibition of β-carotene bleaching, was carried out as described in Kulisic et al.[20] Synthetic antioxidants (BHA and BHT, see above) were used as positive control, whereas working medium with ethanol was used as blank. All determinations were performed in triplicate.

Low-Density Lipoprotein (LDL) Isolation and Copper-Induced LDL Oxidation. LDL was isolated from three normolipidemic donors by density gradient ultracentrifugation using the fixed angle rotor Ti70 on a Beckman preparative ultracentrifuge as previously described.[21] To avoid LDL oxidation during its isolation, EDTA (1 g/L) was present in all steps of the process and all of the buffers were flushed with argon. The purity of the LDL fraction was checked by electrophoresis using a Radiophor electrophoresis system with lipidophor agar medium. Protein concentration was measured according to the method of Lowry et al.[22] LDL concentration was calculated using albumin as reference protein. Prior to copper-induced oxidation, LDL was dialyzed exhaustively overnight against a 200-fold volume of 0.01 M PBS (phosphate buffer saline, 0.9% NaCl, pH 7.4), without EDTA in the argon atmosphere. Samples prepared were LDL in the absence and presence of 0.005, 0.02, and 0.05 g/L of aqueous infusions. Copper-induced oxidation of these LDL samples (0.1 μM) was triggered at 37 °C by 2.5 μM $CuSO_4$ under aerated conditions. The effect of aqueous infusions on LDL oxidation was followed using a Varian Cary 50 UV−vis spectrophotometer. The increase in the absorbance at 234 nm, indicative of conjugated dienes formation, was followed during the copper-induced oxidation. A kinetic study of the time course of the LDL oxidation process in terms of the lag, propagation, and decomposition phase was performed according to an established protocol.[23,24] In both cases of LDL oxidation processes, absorbances at 234 nm at each time point of oxidation were normalized with respect to the absorbance at the beginning of oxidation. All experiments were repeated three times.

In Vitro Digestion Procedure. An in vitro digestion method was carried out using the combination of two methods.[18,24] The model consisted of two phases: a gastric and an intestinal stage. The samples were brought to pH 2.5 with 4 M hydrochloric acid. After addition of pepsin (315 U/mL, Sigma), the mixture was incubated in a shaking water bath at 180 rpm for 2 h at 37 °C. The reaction was stopped by heating at 90 °C for 10 min to inhibit enzymatic activity. The acidity of the mixture was titrated to 7.5 with 0.9 M $NaHCO_3$, and the digest was further incubated at 37 °C for 2.5 h with a pancreatin−bile mixture (4 mg/mL pancreatin, 25 mg/mL bile in 0.1 M $NaHCO_3$). The reaction was stopped by heating at 90 °C for 10 min to inhibit enzymatic activity. This process was performed in duplicate. Digested samples were kept freeze-dried at −80 °C until analyses.

High-Performance Liquid Chromatography/UV−Vis Diode Array Spectroscopy/Electrospray Ionization−Mass Spectrometry (HPLC/UV-Vis-DAD/ESI-MS). Caper and sea fennel metabolites were evaluated by HPLC/UV-vis-DAD/ESI-MS using a Waters instrument (Waters Italia, Milano, Italy) consisting of a 1525 binary HPLC pump, a PDA 996 photodiode array detector, and a Micromass ZQ single-quadrupole mass analyzer equipped with an ESI Z-spray source; analyses were run in negative ion mode under the following conditions: capillary voltage, 2.75 kV; extractor voltage, 2 V; cone voltage, −20 V (and −50 V); source temperature, 150 °C; desolvation temperature, 250 °C; gas (nitrogen) flow, 400 L/h for desolvation and 210 L/h for the cone. Chromatographic runs were performed using a reverse-phase column (Luna C_{18}, 250 × 4.6 mm, 5 μm particle size, Phenomenex Italia). Caper and sea fennel phenolics were eluted with the following gradient of B (acetonitrile) in A (formic acid, 2.5% solution in water): t (min) = 0, 5% B; t =10, 15% B; t = 30, 25% B; t = 35, 30% B; t = 50, 55% B; t = 55, 90% B; t = 57, 100% B, followed by 8 min of isocratic elution at 100% B. The solvent flow rate was 1 mL/min, and the injector volume selected was 20 μL; the temperature was kept at 25 °C with a column oven (Hitachi L-2300, VWR International, Milano, Italy). Collected data were processed through Mass Lynx processing system v. 4.0. Quantification was carried out at 330 nm for mono- and dicinnamoylquinic acids using chlorogenic acid ($R_2 = 0.9999$) as external standard, whereas rutin was used to establish the calibration curve for flavonol glycosides ($R_2 = 0.9998$). Analyses were carried out in triplicate.

Statistical Analysis. Data from each experiment have been treated by two-way analysis of variance (ANOVA) with Bonferroni post-test to determine significant differences in data of various groups. P values of <0.05 were considered to be significant. The software used was SigmaStat version 3.0 (SPSS Science Software, Erkrath, Germany).

RESULTS AND DISCUSSION

Phenolic Composition and Antioxidant Activity of Aqueous Infusions. HPLC/UV-vis-DAD/ESI-MS identification of phenolic compounds present in wild-grown caper and sea fennel from Croatia is reported for the first time. The dominant presence of rutin as well as kaempferol derivatives in caper

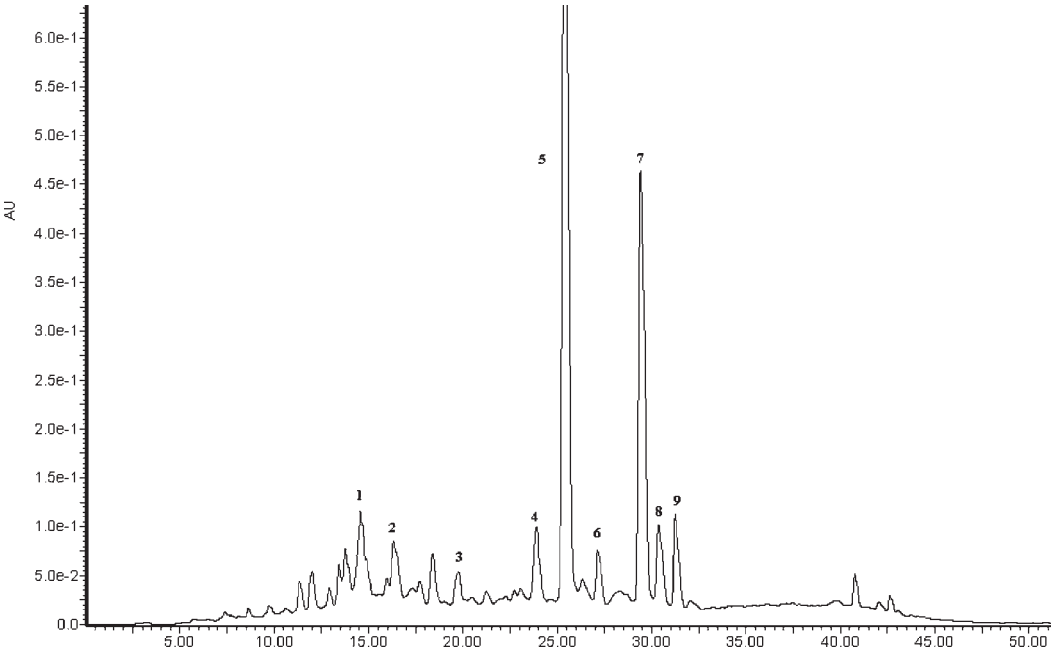

Figure 1. Phenolic compounds identified in caper infusion before in vitro digestion (visualized at 330 nm). Peaks: (1) 5-caffeoylquinic acid; (2) 4-caffeoylquinic acid; (3) 5-*p*-coumaroylquinic acid; (4), 4-feruloylquinic acid; (5) rutin (main peak); (6) quercetin-3-*O*-glucoside; (7) kaempferol-3-*O*-rutinoside; (8) isorhamnetin-3-*O*-rutinoside; (9) kaempferol-3-*O*-glucoside.

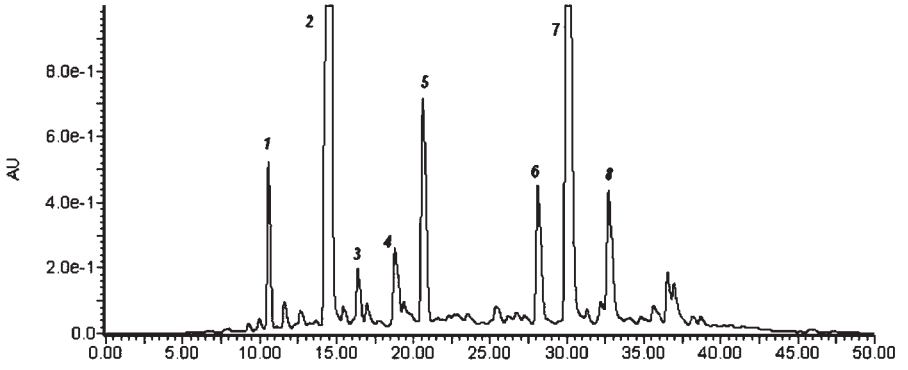

Figure 2. Phenolic compounds identified in sea fennel infusion before in vitro digestion (visualized at 330 nm). Peaks: (1) 3-caffeoylquinic acid; (2) 5-caffeoylquinic acid (chlorogenic acid, main peak); (3) 1-caffeoylquinic acid; (4) 5-*p*-coumaroylquinic acid; (5) 5-feruloylquinic acid; (6) 3,4-dicaffeoylquinic acid; (7) 3,5-dicaffeoylquinic acid; (8) 4,5-dicaffeoylquinic acid.

extracts is documented in several studies.[5,25−29] The presence of chlorogenic acid (5-caffeoylquinic acid) as the major phenolic compound in sea fennel is documented by others.[11,12,30] Phenolics present in the *C. spinosa* aqueous infusion belong to two different classes of secondary metabolites: cinnamoylquinic acids (chromatographic signals between 14 and 25 min) and flavonoids, with higher retention times (from 25 to 32 min), representing the majority of the phenolics in the extract (Figure 1). Unambiguous identification of chromatographic signals was made through their UV−vis and ESI-MS spectra. For flavonoids, particularly, *m*/*z* values of 285, 301, and 315, extracted from total ion current (TIC) chromatograms acquired in negative mode, can be related to the presence in the extract of highly hydroxylated flavonols such as kaempferol, quercetin, and isorhamnetin, respectively. Indeed, rutin (quercetin 3-*O*-rutinoside) is the most abundant flavonol in the infusion, covering nearly half of the total amount of phenolics, followed by kaempferol 3-*O*-rutinoside (Figure 1). Peak assignments were further confirmed through the use of external standards. In the group of cinnamoylquinic acids four peaks were identified as 5-caffeoylquinic acid (chlorogenic acid), 4-caffeoylquinic acid, 5-*p*-coumaroylquinic acid, and 4-feruloylquinic acid on the basis of their diagnostic mass spectra and fragmentations, also according to the works of Clifford et al.;[31,32] some other minor peaks in the same chromatographic area were detected in negligible amounts; their relative retention times, together with their corresponding UV−vis and mass spectra, suggested they may belong to the class of cinnamoylquinic acid derivatives.

The chromatogram of sea fennel aqueous infusion analysis can be similarly divided into two parts (Figure 2): a first one, from 5 to 25 min, is characterized by the elution of monocinammoylquinic acids; a second part, from 25 to 40 min, is when the elution of

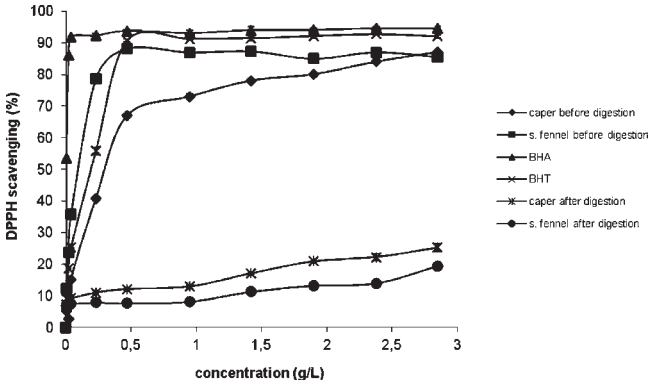

Figure 3. Radical scavenging ability of commercial antioxidants (BHA and BHT) and untreated and digested caper and sea fennel aqueous infusions detected by DPPH method. Values represent the mean value ± SD of three independent experiments. ANOVA with Bonferroni post-test showed that the studied infusions differed significantly ($P \leq 0.05$) in all concentrations used.

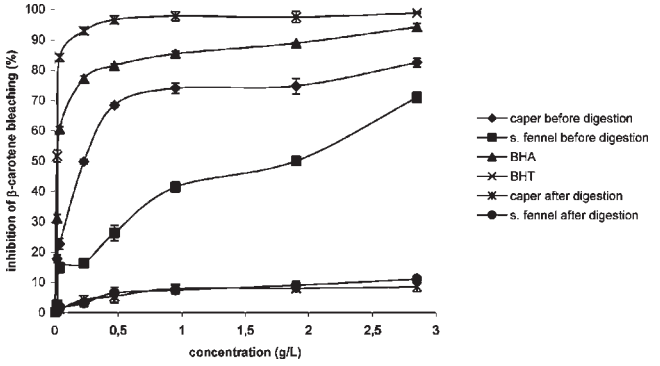

Figure 4. Inhibition of β-carotene bleaching by commercial antioxidants (BHA and BHT) and untreated and digested caper and sea fennel aqueous infusions. Values represent the mean value ± SD of three independent experiments. ANOVA with Bonferroni post-test showed that the studied infusions differed significantly ($P \leq 0.05$) in all concentrations used.

dicinnamoylquinic acids takes place. As discussed for the caper aqueous infusion, MS data were crucial in assigning peak identities; in fact, extraction of the m/z 191 value, belonging to the quinic acid anion as $(M - H)^-$, led to eight different chromatographic signals. Further extraction of a series of pseudomolecular ions at mass values of 353 (caffeoylquinic acid anion), 337 (*p*-coumaroylquinic acid), 367 (feruloylquinic acid), and 515 (dicaffeoylquinic acid), respectively, allowed distinguishing between cinnamoyl- and dicinnamoylquinic acid derivatives. The correct isomeric assignment was made on the basis of the main fragments present in the mass spectra, according to the work of Clifford et al.[31,32]

Results presented in Figure 3 demonstrate a very strong and dose-dependent DPPH scavenging ability by sea fennel infusion (88% at a concentration of 0.4 g/L in the reaction system), which was comparable with those of commercial antioxidants (BHT and BHA). Sea fennel radical scavenging ability may be in relation to the dominant presence of the chlorogenic acid, which is the most common member of the hydroxycinnamic acids and of which the antioxidant properties are well-known.[33,34] High radical scavenging activity (using ABTS radical) of sea fennel was shown by Meot-Duros et al.[30] The radical scavenging ability of

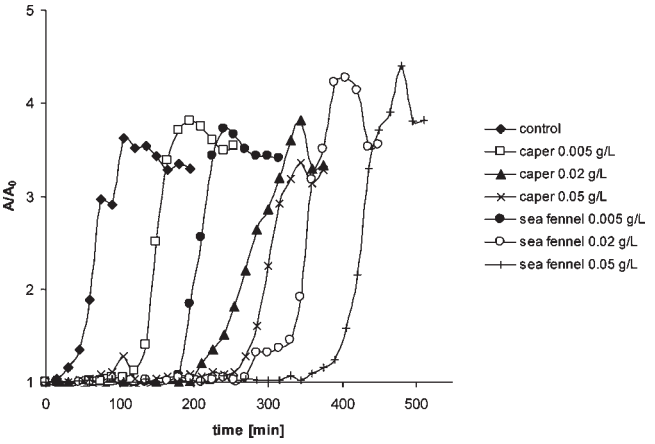

Figure 5. Inhibition of copper-induced oxidation of human LDL by caper and sea fennel infusions. Values represent the mean value of three independent experiments. ANOVA with Bonferroni post-test showed that the studied infusions differed significantly ($P \leq 0.05$) in all concentrations used.

caper is documented in several studies, being explained by the dominant presence of phenolic compounds, such as rutin and kaempferol 3-*O*-rutinoside.[5,28,35] It is known that radical scavenging activity by the DPPH method depends on the number and substitution pattern of −OH groups.[36−38] In that sense, good radical scavenging activity of caper and sea fennel aqueous infusions was expected because both infusions are good sources of polyhydroxylated flavonols. In the β-carotene bleaching method (coupled oxidation of β-carotene and linoleic acid), aqueous infusions from both plants exhibit dose-dependent antioxidant activity in emulsion, which was 13−30% lower than the antioxidant activities of BHA and BHT (Figure 4). The activity of the caper infusion was stronger than that of the sea fennel infusion, the difference being significant.

The third method used in this study to estimate antioxidant activity was copper-induced oxidation of human LDL.[39] Both tested aqueous infusions showed high and dose-dependent ability to inhibit the copper-induced oxidation of human LDL. At the concentration of 0.005 g/L in the reaction system, the prolongation of the lag phase was 122 ± 3 min for the caper infusion and 177 ± 3 min for the sea fennel infusion (Figure 5). At the concentration of 0.02 g/L the lag phase was prolonged for 213 ± 4 min by caper infusion and for 297 ± 14 min by sea fennel infusion. At the highest tested concentration (0.05 g/L in the reaction system), sea fennel prolonged the lag phase for 381 ± 9 min, whereas this prolongation was 263 ± 5 min by caper aqueous infusion. Caper and sea fennel infusions differed significantly ($P \leq 0.05$) in all concentrations used. Rutin and chlorogenic acid, dominant phenolic compounds in caper and sea fennel aqueous infusions, have been shown to inhibit the oxidative modification of LDL in vitro.[40−43]

Phenolic Composition and Antioxidant Activity of Digested Infusions. To investigate the stability of the dominant phenolic compounds from caper and sea fennel aqueous infusions under gastric and intestinal conditions, a two-step in vitro digestion procedure was applied., which has been successfully employed for the study of various foods containing phenolic compounds.[15] According to the FC assay, the amount of total phenols in digested infusions significantly decreased (<1% in both samples), suggesting that the dominant phenolic components detected in original samples, are not stable under the gastrointestinal

Table 1. Effect of Two-Step in Vitro Digestion on Commercial Standard Mixture of Phenolics

peak	compound	standards mix intact[a] (μg/mL)	standards after pepsin treatment (μg/mL)	pepsin loss[b] (%)	standards mix after pancreatin/bile treatment (μg/mL)	loss pancreatin/bile[c] (%)	standards mix after total digestion (μg/mL)	total loss (%)
1	chlorogenic acid	67.50	28.29	58.10	3.26	88.50	2.90	95.70
2	rutin	45.00	5.30	88.11	nd	total	nd	total
3	quercetin 3-*O*-glucoside	30.00	nd	total	nd	total	nd	total
4	quercetin	15.00	nd	total	nd	total	nd	total

[a] For further details on the preparation of the standard mixture, see Materials and Methods. [b] Referred to the difference between the initial standard area and the area after pepsin treatment. [c] Referred to the difference between the pepsin treatment and the pancreatin/bile treatment areas.

Table 2. Effect of Two-Step in Vitro Digestion on Caper Infusion

peak	compound	caper infusion before digestion[a] (μg/mL)	caper infusion after pepsin (μg/mL)	pepsin loss (%)	caper infusion after pancreatin/bile treatment (μg/mL)	further panctreatin/bile loss (%)	caper infusion after total digestion (μg/mL)	total loss (%)
1	5-caffeoylquinic (chlorogenic) acid	3.31	3.12	5.8	2.24	28.0	2.21	33.0
2	4-caffeoylquinic acid	1.84	1.81	1.5	1.35	24.9	1.34	26.4
3	5-*p*-coumaroylquinic acid	0.50	0.48	3.9	0.37	22.1	0.37	25.7
4	4-feruloylquinic acid	0.66	0.64	2.5	0.53	17.2	0.52	19.8
5	rutin	10.20	10.00	1.7	1.68	83.2	nd	total
6	quercetin 3-*O*-glucoside	0.15	0.14	3.8	0.03	77.8	nd	total
7	kaempferol 3-*O*-rutinoside	2.32	2.20	5.0	nd	total	nd	total
8	isorhamnetin 3-*O*-rutinoside	0.82	0.80	2.3	0.11	86.2	nd	total
9	kaempferol 3-*O*-glucoside	0.60	0.56	6.6	nd	total	nd	total

[a] From a 60 g/L initial solution, see Materials and Methods.

Table 3. Effect of Two-Step in Vitro Digestion on Sea Fennel Infusion

peak	compound	sea fennel before digestion[a] (μg/mL)	sea fennel after pepsin (μg/mL)	loss (%)	sea fennel after pancreatin/bile treatment (μg/mL)	loss (%)	sea fennel after total digestion (μg/mL)	total loss (%)
1	3-caffeoylquinic acid	12.00	nd	total	nd	total	nd	total
2	chlorogenic acid	198.60	67.5	66.0	39.1	42.3	36.3	81.7
3	1-caffeoylquinic acid	24.30	nd	total	nd	total	nd	total
4	5-*p*-coumaroylquinic acid	16.55	5.4	67.4	3.67	32.8	3.62	78.1
5	5-feruloylquinic acid	24.98	nd	total	nd	total	nd	total
6	3,4-dicaffeoylquinic acid	15.60	nd	total	nd	total	nd	total
7	3,5-dicaffeoylquinic acid	57.05	nd	total	nd	total	nd	total
8	4,5-dicaffeoylquinic acid	29.60	nd	total	nd	total	nd	total

[a] From a 60 g/L initial solution, see Materials and Methods.

conditions here applied. These data are in agreement with results of other authors, who reported a dramatic decrease of the concentration of selected phenolic compounds (such as rutin and kaempferol) in digesta samples in comparison with the undigested ones.[15,16] On the contrary, some authors reported that gastric digestion had no effect on flavonoid stability.[44,45] According to Friedman and Jürgens,[46] chlorogenic acid (the main phenolic compound present in sea fennel infusion, see Figure 2 and Table 3) is not stable at high pH values (small intestine conditions). It was also found that pepsin digestion had a significant effect on the composition of chlorogenic acid derivatives, decreasing their concentration.[44] Other authors suggest that, during the pancreatic incubation, some isomerization between neochlorogenic acid and chlorogenic acid may occur.[45]

The presented results showed that a general degradation takes place during the in vitro digestion procedure, even if strongly dependent on the matrix. Tables 1−3 show the effects of gastric and pancreatic digestion on a commercial standard mixture of phenolics and caper and sea fennel infusions (the latter both 60 g/L, see Materials and Methods), respectively. In the mix of commercial standards, 5-caffeoylquinic (chlorogenic) acid is the only metabolite still present in the final digested sample, even if in small traces (>95% total loss), whereas quercetin 3-*O*-glucoside and quercetin are totally degraded right after the first step. With regard to the sea fennel infusion matrix, the dicaffeoylquinic acids are completely degraded after the gastric step; chlorogenic acid and 5 *p*-coumaroylquinic acid, on the other hand, lose 82 and

78%, respectively, after the whole digestion process (66 and 67% after the gastric step). The behavior of the cinnamoylquinic acids in the caper infusion greatly differs from the previous matrix; in fact, they encounter losses in the range from ca. 20 to 33% after the whole digestion process, whereas flavonoids are completely degraded. Furthermore, in the same matrix, we observed only negligible losses (from 1.5 to 6.6%) of all the metabolites after the gastric step. Under Phenolic Composition and Antioxidant Activity of Aqueous Infusions, we gave a hint as to how the presence of minor components and, generally, the matrix itself may strongly influence the behavior of single metabolites; this is particularly evident considering the different stabilities of chlorogenic acid in the aforementioned three matrices. As a matter of fact, this metabolite suffers nearly total loss (95.7%) after the whole digestion procedure when part of the standard mixture, a notable 81.7% loss in the sea fennel infusion, and a 33% loss when the caper infusion was involved. Similarly, the behavior of rutin toward the first (gastric) step varies from 88% loss in the standard mixture to a negligible 1.7% loss with the caper infusion.

It appears from the aforementioned analytical data that the "caper matrix" exerts a sort of "self-protection" effect on its components when submitted to the digestion process. Some hypotheses can be formulated on the basis of its composition (especially the so-called minor components, tentatively identified as further cinnamoylquinic acid derivatives), when the "caper matrix" behavior is compared with that of standard mix and/or the sea fennel one. The main difference between the two polyphenolic extracts is the presence of flavonoids in caper, which were not found in reasonable amounts in sea fennel (Tables 2 and 3). On this basis, a protective role of flavonoids toward the other metabolites (on the behalf of their red-ox potential and/or their intrinsic expendability due to a different thermodynamic and/or kinetic behavior) seems possible. One can argue that there are flavonoids in the standard mixture, and still the behavior is not the same, but the ratios among the different components are not the same. In the in vitro digestion procedure used in this study, the activity of digestion enzymes was stopped after each digestion phase (gastric and intestinal) by heating samples at 90 °C for 10 min. To our knowledge, no degradation occurs by keeping the samples at this moderately high temperature for such a short time; however, to verify the stability of the metabolites to these conditions, samples of caper and sea fennel were maintained at 90 °C for 1 h and then analyzed. We concluded that the temperature does not affect the metabolomic profile. This is in agreement with the literature, where flavonoids in particular are considered to be stable during heating.[47] Consequently, DPPH scavenging ability as well as the inhibitory activity on β-carotene bleaching by caper and sea fennel infusions completely decreased after simulated digestion (Figures 3 and 4). In addition, their inhibitory effect on copper-induced oxidation of human LDL disappeared (data not shown). The presented results are in line with those of several studies which show that phenolic compounds are highly sensitive to the mild alkaline conditions in the small intestine and that their structural modifications result from the change in their antioxidant activity.[15,45,48]

The results of this study show that phenolic compounds, identified in caper and sea fennel infusions, are not stable under the applied simulated two-phase gastrointestinal conditions (stomach and small intestine) and that the antioxidant activity of both aqueous infusions should be strongly compromised after in vitro digestion processes. Furthermore, given the behavior of metabolites toward simulated digestion strongly depends on the matrix, the caper infusion was demonstrated to possess the strongest "protection" ability toward its components.

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Funding Sources

T.K.-B. was granted a postdoctoral fellowship of the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia.

ACKNOWLEDGMENT

We are grateful to Dr. Ivica Blazevic for critical reading of the manuscript. We also thank Tonia Strano (ICB-CNR, Catania) for her skillful technical assistance.

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