Investigation of Antioxidant and Rosmarinic Acid Variation in the Sage Collection of the Genebank in Gatersleben

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The total phenolic and flavonoid contents and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reduction antioxidant power (FRAP) antioxidant capacities of 19 accessions of *Salvia officinalis* from the sage collection of the genebank in Gatersleben (Germany) were evaluated. The major phenolic compounds of sage, that is, rosmarinic acid, caffeic acid, carnosol, and carnosic acid, were quantified by reverse-phase high-performance liquid chromatography. The aerial parts of different individual plants of each accession were collected in two consecutive years from the same experimental field at the beginning of their flowering period. The results demonstrated a high variability between accessions. A general decreasing tendency from 2007 to 2008 was observed in most of the estimated parameters, that is, total phenolic, total flavonoid, rosmarinic acid, and caffeic acid contents and DPPH antioxidant activity. A slight opposite trend was obtained with the FRAP antioxidant capacity. Low but variable quantities of carnosol and carnosic acid were evaluated in the sample extracts. Individual plants within accessions were identified with high phenolic content and strong antioxidant activity. The rosmarinic acid content showed up to 8-fold differences between the lowest and the highest values. Overall, the study demonstrated a high variability in secondary metabolites present in sage, which could be used for breeding of highly antioxidative genotypes of *S. officinalis*.

KEYWORDS: Antioxidant capacity; rosmarinic acid; carnosic acid; carnosol; caffeic acid; *Salvia officinalis*; sage

INTRODUCTION

Salvia officinalis L. (sage, Lamiaceae) is one of the typical herbs used from earliest times for flavoring, improving, preserving, and diversifying everyday foods. Sage has been used in Europe for centuries as a spice and is well-known as a medicinal plant and potent natural antioxidant of the common aromatic plants (*1*). The main chemical components identified in sage are essential oil (1.5−3%), hydroxycinnamic derivatives (3.5%), phenolic diterpenes, triterpenes, flavonoids, phenolic glycosides, polysaccharides, and others constituents, including small amounts of benzoic acid derivatives and phytosterols (*2−6*). A number of studies have reported the antioxidant capacity of sage and other *Salvia* species (*7*). Rosmarinic acid, carnosol, carnosic acid, and also some essential oil and flavonoid compounds were identified as the major antioxidants present in sage (*1,4,8*). However the majority of wild-growing *Salvia* species are still being evaluated for their phytochemical compounds and their medicinal properties (*9*).

Extracts from sage and rosemary (*10*) have been proposed for the protection and preservation of certain food and nutraceutical products to avoid the possible toxicity of synthetic antioxidants

(*4,6*). The essential oil and flavourants of sage are used as basic materials for various foods and cosmetic and pharmaceutical preparations.

The chemical and biological diversity of aromatic and medicinal plants depends on various factors such as the location of the cultivation, climatic conditions, vegetative phase, genetic outfit, and others. The species of the Lamiaceae family are known to be important sources of phenolic type pharmaceuticals, and rosmarinic acid is found in substantial quantities in several species of the family (*11*). The plant-to-plant variability of specific metabolites like rosmarinic acid remains a major problem in the use of phytopharmaceuticals from the Lamiaceae species (*11*). The variability of rosmarinic acid production using cell suspension cultures of sage and different type of culture medium has been demonstrated (*12*). Another study reported an increase in rosmarinic acid yield in the leaves of *S. officinalis* by phosphorus application (*13*).

The aim of this work was to evaluate the variability of rosmarinic acid, caffeic acid, carnosic acid, carnosol, and some antioxidant parameters [i.e., total phenolics, total flavonoids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reduction antioxidant power (FRAP)] of 19 sage accessions from the genebank Gatersleben (Germany) in two consecutive years. To minimize the variability

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3814 *J. Agric. Food Chem.,* Vol. 58, No. 6, 2010

Lamien-Meda et al.

Table 1. List of *S. officinalis* L. Samples with Their Origins[a]

no.	origin	country
1	Botanical Institute, Sofia	Bulgaria
2	Dalmatia	Croatia
3	Podgorje, between Krasno-Polje and Senj	Croatia
4	Seed Company H. Mette & Co., Quedlinburg	Germany
5	Bot. Garden Univ. Mainz	Germany
6	People-owned enterprise for pharmaceuticals Dresden, Research Centre Artern	Germany
7	Braunschweig Genetic Resources Centre	Germany
8	Braunschweig Genetic Resources Centre	Germany
9	Braunschweig Genetic Resources Centre	Germany
10	Braunschweig Genetic Resources Centre	Germany
11	Braunschweig Genetic Resources Centre	Germany
12	Bot. Garden Univ. Athens	Greek
13	Bot. Garden Univ. Budapest	Hungary
14	Island Tremiti, S. Domino, "Carducci Amerago" Farm	Italy
15	Applied Botany of Pyongyang	North Korea
16	Agro-Bot. Garden. Cluj	Rumania
17	Agro-Bot. Garden Cluj	Rumania
18	Rudník, SW St. Turá, Biele Karpaty	Slovakia
19	Redwood City Seed Co., California	United States

[a] Samples are provided from the complete *S. officinalis* L. collection of the gene-bank of Gatersleben without any sorting criteria.

due to the locality and those induced by the environmental, physiological, and morphological factors, the sage collection was cultivated in the same location and harvested in two consecutive years at the beginning of the flowering stage. The variability of the essential oil composition (*14*) of the same sage collection has been studied.

MATERIALS AND METHODS

Plant Material and Extractions. Nineteen accessions of *S. officinalis* L. from the genebank collection of Gatersleben were cultivated at the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben (Germany) in 2006. In the first year (2007), the aerial parts of 10 individual plants of each of the 19 accessions were collected, and in the second year (2008), the collections from the same culture were grouped into three samples per accession (meaning three individual plants per sample). The collected samples were air-dried at room temperature in a drying chamber (20−25 °C, 20−30% relative humidity) before their use for analysis. Within the 19 accessions, eight were from Germany (#4−#11), two were from Rumania (#16 and #17) and Croatia (#2 and #3), and one was from North Korea (#15), the United States (#19), Bulgaria (#1), Greece (#12), Hungary (#13), Italy (#14), and Slovakia (#18), respectively (**Table 1**). The genetic structures of the 19 accessions were studied, and 51 and 49% of variance were found between and within the populations, respectively (*15*).

Two hundred milligrams of ground plant material was extracted with 32 mL of 50% aqueous methanol for 60 min in a cooled ultrasonic bath (*13*). The filtered extracts were aliquoted in 2 mL eppendorf tubes and kept at −20 °C until analysis.

Chemicals and Reagents. Folin−Ciocalteu reagent, methanol (p.a.), sodium nitrite (p.a., ≥99%), sodium acetate trihydrate (p.a.), and ferric chloride hexahydrate (p.a.) were purchased from Merck (Darmstadt, Germany). DPPH radical (DPPH•, 98%), aluminum trichloride (≥98%), 2,4,6-tripyridyl-*s*-triazine (TPTZ, ≥99%), carnosol, caffeic acid, and (+)-catechin were obtained from Sigma-Aldrich (Steinheim, Germany). Sodium carbonate anhydrous, sodium hydroxide (p.a.), hydrochloric acid (32%, p.a.), glacial acetic acid (100%, p.a.), acetonitrile, high-performance liquid chromatography (HPLC) grade methanol, rosmarinic acid, and carnosic acid were purchased from Carl Roth (Karlsruhe, Germany). Trolox was from Fluka (Denmark, Germany).

Total Phenolics and Total Flavonoids. The Folin−Ciocalteu reagent was used to determine the total phenolic content (*16*). The sample extract (40 μL in 2 mL of H_2O) was mixed with 100 μL of 2 N Folin−Ciocalteu reagent. This mixture was kept at room temperature for 3 min, and then,

200 μL of sodium carbonate solution (35 g in 100 mL of H_2O) was added. The final volume was filled up to 5 mL with H_2O. After 60 min of incubation in the dark, the absorbance was measured at 725 nm against a water blank using a spectrophotometer (Hitachi 150-20, Tokyo, Japan). A calibration curve was plotted using caffeic acid ($y = 0.0211x$, $r^2 = 0.9997$). Determination was performed in triplicate, and results were expressed as mg of caffeic acid equivalents (CAE) per gram of plant dry weight (DW) and are presented as means of triplicate analyses.

The total flavonoid content was determined using the aluminum chloride colorimetric assay (*17*). Briefly, 250 μL of sample extract was diluted with 1.25 mL of H_2O and mixed with 75 μL of 5% $NaNO_2$ and 150 μL of 10% $AlCl_3 \cdot 6H_2O$ for 5 min. Then, 500 μL of 1 M NaOH and 275 μL of H_2O were added. The absorbance of the reaction mixture was measured at 510 nm. (+)-Catechin was used as a standard to produce the calibration curve ($y = 0.0951$, $r^2 = 0.9982$). The means of three readings were used, and the total flavonoid content was expressed in mg of catechin equivalents (CE) per gram DW and are presented as means of triplicate analyses.

DPPH Radical Scavenging Activity. The radical scavenging activity of the sample extracts for the radical DPPH was measured as described (*16*) with some modifications. The 50% methanolic extracts (5 μL) were diluted to 100 μL with methanol and mixed with 100 μL of DPPH solution (0.015%). After incubation at room temperature in the dark for 30 min, the absorbance of the reaction mixture was measured at 490 nm using a plate reader (BIO-RAD 450, Tokyo, Japan). Trolox was used as the standard for the calibration curve ($y = -0.1985x + 0.7551$, $r^2 = 0.9979$). A blank consisting of a high concentration of trolox was used to correct all readings. The results were expressed in mg trolox equivalents (TE) per gram DW and are presented as means of triplicate analyses.

FRAP. The ability of the extract to reduce ferric TPTZ to a colored product was assessed by the method of ref *18*. A working reagent was prepared by mixing 25 mL of acetate buffer (pH 3.6) with 2.5 mL of 10 mM TPTZ in 40 mM HCl and 2.5 mL of freshly prepared solution of 20 mM $FeCl_3 \cdot 6H_2O$. For the assay, 6 μL of sample extract was diluted with 18 μL of H_2O and mixed with 180 μL of working reagent. After 4 min of incubation, the absorbance was measured at 595 nm using a plate reader. Trolox was used as a standard ($y = 0.4657x$, $r^2 = 0.9915$), and the results were expressed as mg TE per gram DW and are presented as means of triplicate analyses.

HPLC Analysis of Carnosol, Caffeic, Rosmarinic, and Carnosic Acids. The HPLC analyses were performed using a Waters (Milford, MA) chromatograph equipped with a quaternary pump, a photodiode array detector, and an autoinjector. An Empower software Build 1150 from Waters cooperation was used for data collection and processing. Separations were performed on a reverse phase Symmetry column (4.6 mm × 150 mm, 5 μm pore size) equipped with a Symmetry C18 guard column (Waters). A gradient elution was carried out at a flow rate of 1.5 mL/min using 1% acetic acid:acetonitrile (85:15 v/v) for solvent A and methanol for solvent B. The analysis started with a 10% solvent B, and a linear gradient was performed to reach 100% solvent B within 30 min. From 30 to 40 min, solvent B was kept constant at 100% (*13*).

The identification of the phenolic compounds was done by comparing the retention times and the UV spectra obtained by diode array detector of unknown peaks to those of reference chromatography standards. The detection was performed at 330 nm for rosmarinic acid and caffeic acid and at 285 nm for carnosol and carnosic acid.

The quantification was done by comparison with an external standard of rosmarinic acid ($y = 50.499x$, $r^2 = 0.9997$), carnosic acid ($y = 1.8094x$, $r^2 = 0.9964$), and caffeic acid ($y = 60.274x$, $r^2 = 0.9960$) and expressed as mg per gram of DW. Standard curves were made from each standard at the concentrations of 1−500 μg/mL except carnosol, which was quantified in carnosic acid equivalent.

Statistics. The statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL). Considering the difference between the two years of sampling (10 per accession the first year and 3 per accession the second year), the mean values of all of the studied parameters except carnosic acid and carnosol contents were compared using graphs. Boxplot draws of the first year data were used to show the variability between and within accessions. All of the data were subjected to analysis of variance to estimate the variability between and within accessions. Pearson correlations between the studied parameters were also estimated.

Article

J. Agric. Food Chem., Vol. 58, No. 6, 2010   **3815**
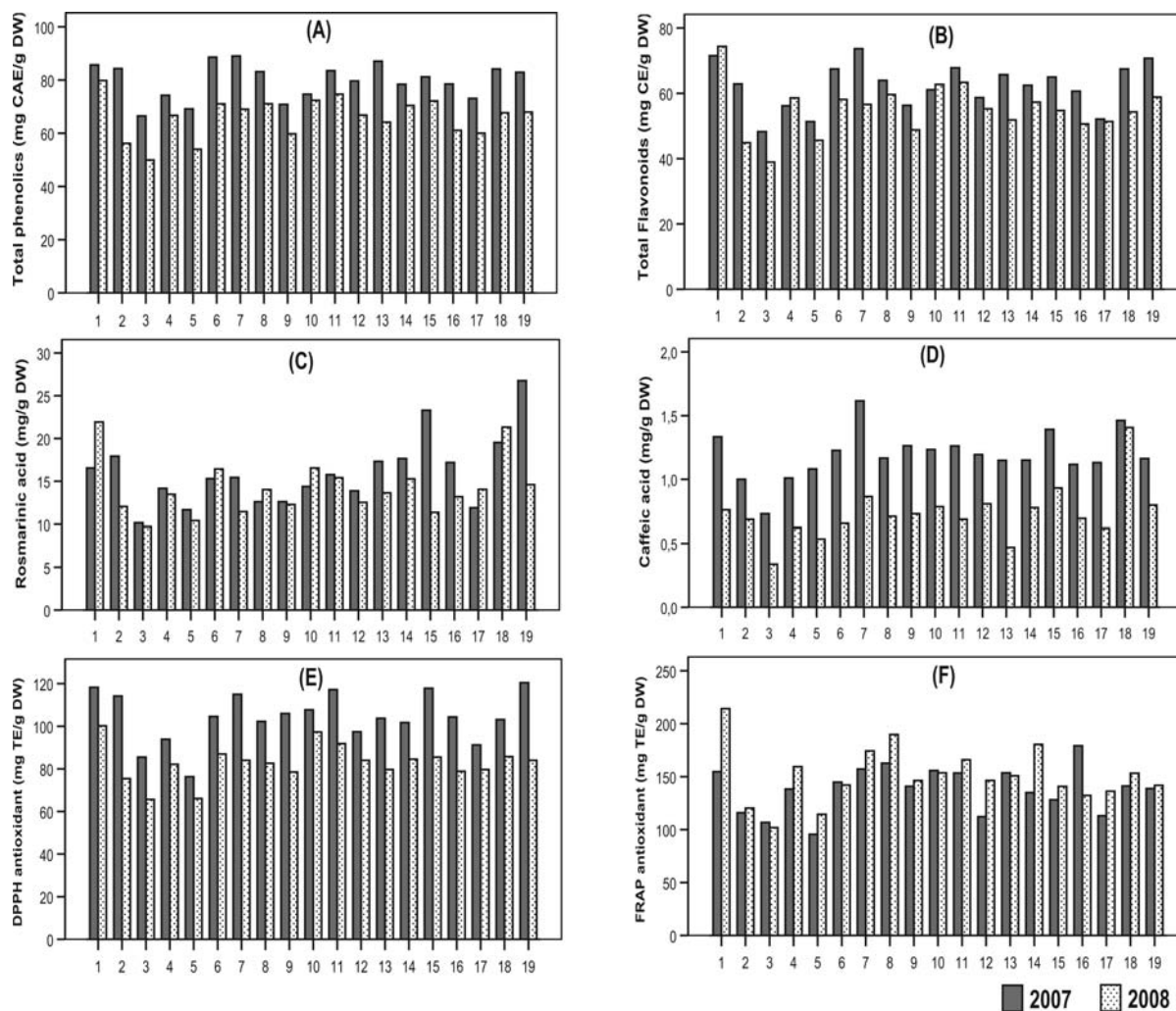
Figure 1. Comparative mean values of total phenolics (**A**), total flavonoids (**B**), rosmarinic acid (**C**), caffeic acid (**D**), DPPH antioxidant (**E**), and FRAP antioxidant (**F**) of *S. officinalis* collections from 2007 and 2008.

RESULTS AND DISCUSSION

Phenolics. The total phenolics and total flavonoid contents were analyzed spectrophotometrically using caffeic acid and catechin as standards, respectively. The total phenolics content varied from 67 to 89 mg CAE/g DW in 2007 and from 50 to 80 mg CAE/g DW in 2008 (**Figure 1A**). The total flavonoids ranged from 48 to 74 mg CE/g DW in 2007 and from 40 to 74 mg CE/g DW in 2008 (**Figure 1B**). The mean total phenolic and total flavonoid contents of the accessions #3, #5, #9, and #17 were significantly lower as compared to the other accessions for both years. The Croatian accession (#3) had the lowest phenolic and flavonoid contents for both years, and accessions from Germany (#7) and Bulgaria (#1) had the highest values for 2007 and 2008, respectively. Tukey's test ($P < 0.05$) of the total phenolics and total flavonoids showed significant variability between accessions for 2007 and 2008 data. **Figure 2A,B** presents a slight variability of the total phenolics and total flavonoids within some accessions, for example, #3, #7, and #13. Significantly higher values were observed in the data of 2007 as compared to those of 2008 for the total phenolics and total flavonoids ($P < 0.001$). A high similarity was observed between the total phenolics and the total flavonoids pattern (**Figures 1A,B** and **2A,B**) explaining that both parameters had a similar variability between accessions. This was confirmed by the strong correlation ($r = 0.89$) observed between them. The total phenolic content in this study is higher as compared to

the concentration (39−43 mg CAE/g DW) obtained by Nell et al. (*13*) with *S. officinalis* leaves from a single plant rooting propagation, using the same quantification method. The 1.2−2.1-fold difference could be attributed mainly to the differences between the growth conditions and also to the accession.

Similar total phenolic contents were observed for *S. officinalis* leaves and stems from New Zealand (*19*), *Salvia fruticosa* Miller (*20*), and also for other Lamiaceae plants, that is, *Ocimum basilicum* L. with 23−66 mg of gallic acid equivalent (GAE)/g DW (*19*, *21*), *Origanum* sp. with 70−126 mg GAE/g DW (*19*, *22*), *Rosmarinus officinalis* L. with 42−134 mg GAE/g DW (*19*, *20*, *22*), *Thymus vulgaris* L. with 32−71 mg CAE/g DW (*16*, *19*), and *Mentha* sp. with 51−138 mg GAE/g DW (*19*, *20*). Shan et al. (*19*) found in a study of different spice extracts that the total phenolic content of the six Lamiaceae decreased in the following order: *Origanum vulgare* > *S. officinalis* > *R. officinalis* > *T. vulgaris* > *O. basilicum* > *Mentha canadensis*. On the other hand, the total flavonoid content of this study seems to be higher than those of the Lamiaceae studied by Papageorgiou et al. (*22*), that is, *Mentha viridis* (24.1−27.8 mg of epicatechin equivalent ECE/g DW) > *Origanum majorana* (14.5−28.8 mg ECE/g DW) > *R. officinalis* (traces−25.1 mg ECE/g DW).

The mean results of the phenolics and flavonoids showed that the total flavonoid content represents 71−85% of the total phenolic content. This can be attributed to the nonspecificity of the

3816 *J. Agric. Food Chem.,* Vol. 58, No. 6, 2010

Lamien-Meda et al.
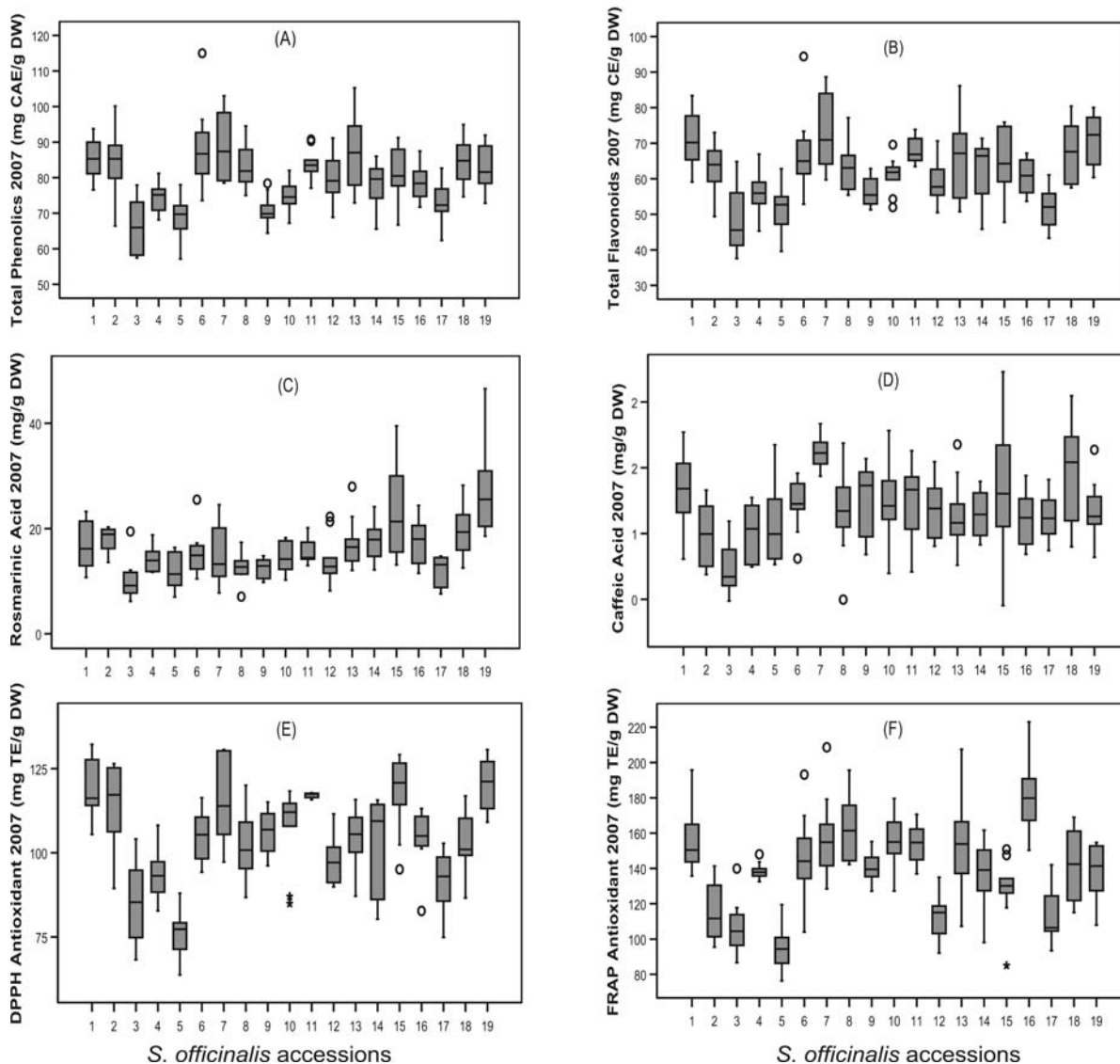
Figure 2. Boxplot graphs of the first year total phenolics (**A**), total flavonoids (**B**), rosmarinic acid (**C**), caffeic acid (**D**), DPPH antioxidant (**E**), and FRAP antioxidant (**F**) of the *S. officinalis* collection.

$AlCl_3$–$NaNO_2$ method used for the flavonoids quantification. Indeed, rosmarinic acid, which is known as the major phenolic compound of sage (*23*), and also caffeic acid showed positive reaction with the $AlCl_3$–$NaNO_2$ reagent. The two compounds presented the same reactivity as the standard compound catechin, with corresponding factors of 1.1 and 1.04 for rosmarinic and caffeic acids, respectively.

Several flavones and flavonols with their glycosides, that is, apigenin, genkwanin, luteolin, scutellarein, and its iso-form, cirsiliol, hesperetin, hispidulin, cirsimaritin, and quercetin have been characterized in *S. officinalis* (*5*, *23*, *24*). Other important phenolic compounds identified in sage are phenolic acids with their glycosides (*5*). Among the sage phenolics, rosmarinic acid has been reported to be the predominant phenolic in many studies (*3*, *7*, *10*, *25*).

Rosmarinic acid can reach up to 3.3% in some sage samples (*3*). The quantitative data of rosmarinic acid and caffeic acid were used in this study to evaluate the variability within and between the 19 sage accessions. The HPLC results of the rosmarinic acid content ranged from 6 to 47 mg/g DW in 2007 and from 5 to 25 mg/g DW in 2008 (**Figures 1C** and **2C**), showing 5–8-fold differences between the lowest and the highest values. In this study,

the mean rosmarinic acid values are similar to those reported for *S. officinalis* (*10*, *13*, *25*–*27*). However, some individual samples within accessions showed significantly high values. The phenolics pattern at 285 and 330 nm of all of the samples did confirm the fact that rosmarinic acid is the predominant phenolic compound in *S. officinalis*. The accession #19 from California possessed the highest rosmarinic acid content in 2007 followed by accession #15 from Pyongyang and #19 from the United States. In 2008, the higher rosmarinic acid values were obtained with accessions #1, #18, and #10 from Bulgaria, Slovakia, and Germany, respectively. Accessions #3 and #5 (from Croatia and Germany, respectively) had the lowest rosmarinic acid values for both years. Comparing rosmarinic acid data of the 2 years, a similar trend was observed from the first year to the second ($p < 0.05$).

The reactivity of rosmarinic acid to the total flavonoid content assay has been determined, and the contribution of rosmarinic acid to the total flavonoid content ranged from 14 to 65%. The correlation between the rosmarinic acid and the total flavonoids ($r = 0.67$, $p < 0.01$) was higher than that between rosmarinic acid and total phenolics ($r = 0.52$, $p < 0.01$). Similar rosmarinic acid contents of 0.2–12.8, 6.3–20.9, 19.1, and 25.6 mg/g DW were observed for *Salvia* sp. (*19*, *20*, *28*), *R. officinalis* (*19*, *20*),

Article

J. Agric. Food Chem., Vol. 58, No. 6, 2010   **3817**
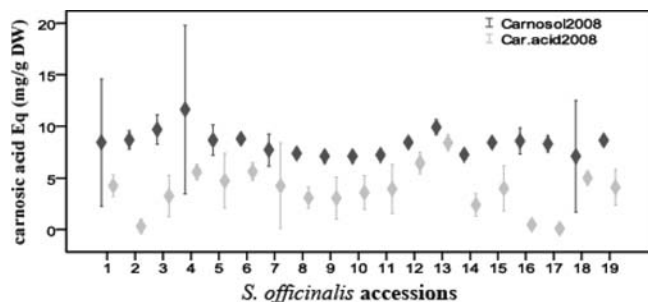
Figure 3. Carnosol and carnosic acid content of the *S. officinalis* collection, with a 95% confidence interval for the mean.

T. vulgaris L. (*16*, *19*), *M. canadensis* L. (*19*), and *O. vulgare* L. (*19*), respectively.

The rosmarinic acid content of the studied sage accessions seemed to be in agreement with the following decreasing order observed by Shan et al. (*19*) with the Lamiaceae species: *O. vulgare* > *S. officinalis* > *M. canadensis* > *R. officinalis* > *O. basilicum* > *T. vulgaris*. However, the genotypic and environmental differences within species, the choice of parts tested, the time of taking samples, and the determination methods make the comparison between data of different studies unclear.

Figures 1D and **2D** showed low values of caffeic acid (0.7−1.6 and 0.3−1.4 mg/g DW for, respectively, 2007 and 2008), confirming the results of other studies (*10*, *25*, *27*). The chromatographic separation of rosmarinic and caffeic acids at 330 nm in a sage sample is presented by **Figure 4A**. Caffeic acid is known to play a central role in the biochemistry of Lamiaceae. The low amount of caffeic acid in the sage samples can be attributed to the fact that caffeic acid is the building block of a variety of the plant metabolites (*5*). In spite of its low quantities, medium correlations were observed between caffeic acid/total phenolics and caffeic acid/total flavonoids ($r = 0.53$ and 0.42, respectively). The 2 years of caffeic acid data were accession-dependent with a decreasing tendency observed from the first year to the second.

The results of the carnosol and carnosic acid quantification (only performed in the second year samples) presented again a high variability between the 19 accessions (**Figure 3**). The chromatographic separation of carnosol and carnosic acid at 285 nm in a sage sample is presented by **Figure 4B**. The lowest amounts of carnosic acid were observed with accessions #2, #16, and #17 (from Croatia and Rumania) and were 20−80-fold lower than the value of accession #13 from Hungary. However, they were in agreement with the average carnosic acid value (2.1 g/kg extract, with an extraction yield of 20.1%) obtained by Pizzale et al. (*26*) with *S. officinalis* samples from Northern Italy. Similar values were observed with sage (*19*, *29*) and *R. officinalis* extracts (*19*). Carnosic acid is a phenolic diterpene that has been identified from sage leaves and showed the greatest antioxidant activity in several models among other diterpenes (*4*, *24*).

The carnosol concentration ranged from 4.1 to 15.1 mg/g DW and showed a weak variability between accessions (**Figure 3**). Carnosol was found to be an oxidation production of carnosic acid, and in turn, carnosol can degrade further to produce other phenolic diterpenes with γ-lactone structures (*8*). Different studies on sage (*8*, *30*) and *R. officinalis* (*31*) using organic extracts have reported a high proportion of carnosic acid comparatively to carnosol. The highest amount of carnosol in this study could be attributed to the type of solvent used. Indeed, 50% methanolic solvent remains favorable for carnosic acid oxidation and is less appropriate for the extraction of sage diterpenic compounds comparatively to dichloromethane. Baskan et al. (*29*) have found with *S. officinalis* tea bags that there was little difference between
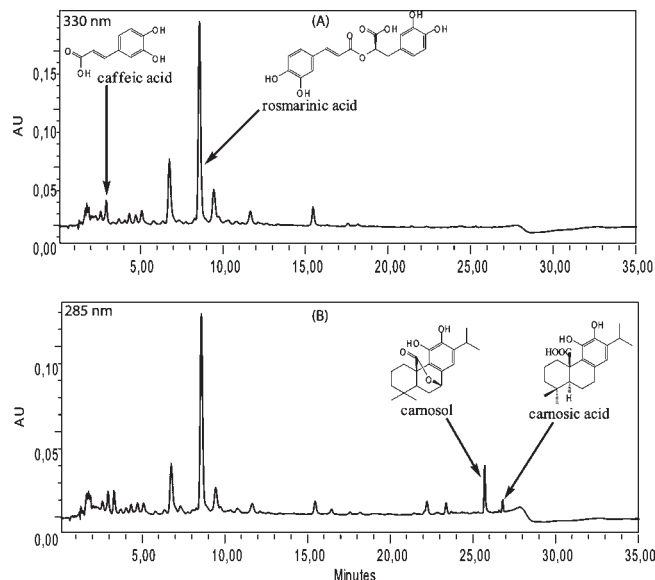
Figure 4. *S. officinalis* chromatogram showing the quantified compounds rosmarinic acid and caffeic acid at 330 nm (**A**) and carnosol and carnosic acid at 285 nm (**B**).

methanol, acetonitrile, and acetone for the solubility of carnosic acid, and also, carnosic acid was not detected in the water extraction solution.

Antioxidant Activity. FRAP and DPPH radical scavenging assays are two common antioxidant assays used for the characterization of plant extracts. FRAP is often used as an indicator of electron-donating activity, and the free radical DPPH reacts with electron donors (*10*). The DPPH and FRAP antioxidant capacities results are shown in **Figures 1E,F** and **2E,F**. The DPPH antioxidant capacity ranged from 64 to 132 mg TE/g DW in 2007 and from 62 to 107 mg TE/g DW in 2008. The FRAP assay varied from 76 to 223 mg TE/g DW in the first year and from 92 to 246 mg TE/g DW in the second year. A high variability of the antioxidant activity between accessions was found (**Figure 2E,F**) with a decreasing trend of the DPPH values from 2007 to 2008 (**Figure 1E,F**). A slight increasing trend of the FRAP activity from 2007 to 2008 was observed in most of the accessions.

In general, all of the accessions in this study had relatively high antioxidant capacities, confirming the strong antioxidant potential of *S. officinalis* (*4*, *24*). The accessions #1 from Bulgaria and #11 from Germany presented high antioxidant activity with both assays for both 2007 and 2008 years. The same accessions also had a high amount of total phenolics and total flavonoids. Likewise, the two accessions #3 and #5 (from Croatia and Germany, respectively) with the lowest DPPH antioxidant activity also had lower FRAP activity and lower total phenolic, total flavonoid, and rosmarinic acid contents. These observations explained the significant correlations observed between the antioxidant activities and the total phenolic ($r = 0.83$ and 0.46, $P < 0.001$, for DPPH and FRAP, respectively) and total flavonoid contents ($r = 0.83$ and 0.62, $P < 0.001$, for DPPH and FRAP, respectively). The rosmarinic acid content correlated well with both DPPH ($r = 0.58$, $P < 0.001$) and FRAP assays ($r = 0.38$, $P < 0.001$). The correlations between the total flavonoids and the two antioxidant assays were stronger than those between the total phenolic content with the DPPH and FRAP. Good correlation between total phenolic analysis and antioxidant assays has been previously reported (*25*, *32*, *33*). The two phenolic diterpenes data from the second year samples, that is, carnosic acid and carnosol, did not correlate with the DPPH and FRAP antioxidant activities.

However, the carnosic acid content of dichloromethane extracts of *R. officinalis* has been proved to be the best predictor of anti-oxidant activity (*31*).

The antioxidant activity of rosmarinic acid has been determined, and the corresponding factor of rosmarinic acid relative to trolox was 1.45 for the DPPH antioxidant and 2 for the FRAP antioxidant activity. The contribution of rosmarinic acid to the different sage antioxidant activity ranged from 11 to 55% for the DPPH antioxidant and from 8 to 62% for the FRAP antioxidant. These contributions are similar to those of *T. vulgaris* extracts described by Chizzola et al. (*16*).

Comparing our sage DPPH and FRAP antioxidant activities to those of *T. vulgaris* studied with exactly the same methods by Chizzola et al. (*16*), *S. officinalis* extracts present 1−2.2-fold and 1.1−3.6-fold higher DPPH and FRAP activities than *T. vulgaris* extracts, respectively. This is in agreement with the following antioxidant decreasing order observed by Shan al. (*19*) with Lamiaceae species: *O. vulgare* > *S. officinalis* > *T. vulgaris* > *R. officinalis* > *M. canadensis* > *O. basilicum*.

The study showed a high variability between and also within the 19 sage accessions through the parameters under study, that is, total phenolics, total flavonoids, rosmarinic acid, caffeic acid, carnosol, carnosic acid, and antioxidant activity. These findings confirmed previous results on the high amount of rosmarinic acid in sage. Among the 19 accessions, some were found with lower phenolics and antioxidant activity, and others presented greater phenolic content and antioxidant activity. The study also confirmed the high antioxidant potential of *S. officinalis* and showed that some interesting sage samples or accessions could be selected for breeding highly antioxidative sage to be used for food conservation.

ABBREVIATIONS USED

DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reduction antioxidant power; TPTZ, 2,4,6-tripyridyl-*s*-triazine; CAE, caffeic acid equivalent; DW, dry weight; TE, trolox equivalent; GAE, gallic acid equivalent; ECE, epicatechin equivalent; CE, catechin equivalent.

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Article

J. Agric. Food Chem., Vol. 58, No. 6, 2010 **3819**

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