Food Chemistry 115 (2009) 650–656

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Chicoric acid found in basil (*Ocimum basilicum* L.) leaves

Jungmin Lee [a,*], Carolyn F. Scagel [b]

[a] United States Department of Agriculture, Agricultural Research Service, PWA, Horticultural Crops Research Unit Worksite, 29603 U of I Lane, Parma, ID 83660, USA
[b] United States Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Unit, Corvallis, OR 97330, USA

ARTICLE INFO

Article history:
Received 28 August 2008
Received in revised form 18 December 2008
Accepted 19 December 2008

Keywords:
Phenolics
Cichoric acid
Caffeic acid derivatives
Dicaffeoyltartaric acid
Lamiaceae
Glomus intraradices

ABSTRACT

This is the first report to identify the presence of chicoric acid (cichoric acid; also known as dicaffeoyltartaric acid, which is a caffeic acid derivatized with tartaric acid) in basil leaves. Rosmarinic acid, chicoric acid and caftaric acid (in the order of most abundant to least; all derivatives of caffeic acid) were identified in fresh basil leaves. Rosmarinic acid was the main phenolic compound found in both leaves and stems. Chicoric acid was not detected in sweet basil stems, although a small amount was present in Thai basil stems. Other cinnamic acid monomers, dimers and trimers were also found in minor quantities in both stems and leaves. Basil polyphenolic contents were determined by blanched methanol extraction, followed by HPLC/DAD analysis. The characterization of the polyphenolics found in the basil extracts were performed by HPLC/DAD/ESI–MS/MS and co-chromatographed with purchased standard. The influence of inoculation with an arbuscular mycorrhizal fungus (AMF), *Glomus intraradices*, on plant phenolic composition was studied on two basil cultivars,'Genovese Italian' and 'Purple Petra'. Inoculation with AMF increased total anthocyanin concentration of 'Purple Petra' but did not alter polyphenolic content or profile of leaves and stems, of either cultivar, compared to non-inoculated plants. In the US diet, basil presents a more accessible source of chicoric acid than does *Echinacea purpurea*, in which it is the major phenolic compound.

Published by Elsevier Ltd.

1. Introduction

Culinary herbs have been reported to possess antioxidant activities (Yanishlieva, Marinova, & Pokorny, 2006) suggesting that they might have potential human health benefits. Basil (family *Lamiaceae*) is a popular herb in the US and Mediterranean diets. Basil's importance as a culinary herb, its historic usage, essential oil composition and phenolics have been well reviewed by Makri and Kintzios (2008). Basil has shown antioxidant and antimicrobial activities due to its phenolic and aromatic compounds (Gutierrez, Barry-Ryan, & Bourke, 2008; Hussain, Anwar, Sherazi, & Przybylski, 2008; Javanmardi, Khalighi, Kashi, Bais, & Vivanco, 2002; Yanishlieva et al., 2006).

The main phenolics reported in basil are phenolic acids and flavonol-glycosides (Javanmardi et al., 2002; Jayasinghe, Gotoh, Aoki, & Wada, 2003; Kivilompolo & Hyotylainen, 2007; Kosar, Dorman, & Hiltunen, 2005; Nguyen & Niemeyer, 2008; Tada, Murakami, Omoto, Shimomura, & Ishimaru, 1996). The presence of caffeic acid derivatives (phenolic acid class) has been reported in basil (Jayasinghe et al., 2003; Kivilompolo & Hyotylainen, 2007; Nguyen & Niemeyer, 2008; Tada et al., 1996; Toussaint, Smith, & Smith, 2007), but complete phenolic profiles of basil have not been reported. Of the caffeic acid derivatives in basil, the present study is the first to identify the presence of chicoric and caftaric acids in basil, respectively the second and third major phenolic acids present in basil leaves.

Chicoric acid is the dominant phenolic reported in *Echinacea purpurea* (Molgaard, Johnsen, Christensen, & Cornett, 2003; Perry, Burgess, & Glennie, 2001) and has been found in all parts (flower heads, leaves, stems and root) of the *E. purpurea* plant (Molgaard et al., 2003). *Echinacea* (family *Asteraceae*) has been reported to have potential antioxidant, anti-inflammatory, antiviral and immunostimulating properties, arising from the naturally occurring alkamides, caffeic acid derivatives, polysaccharides and glycoproteins (Barnes, Anderson, Gibbons, & Philipson, 2005; Charvat, Lee, Robinson, & Chamberlin, 2006; Dalby-Brown, Barsett, Landbo, Meyer, & Molgaard, 2005; Molgaard et al., 2003; Perry et al., 2001). However, in a well-summarized and detailed review of *Echinacea* species' possible health benefits, its effectiveness was no better than that of a placebo (Barnes et al., 2005 and references therein).

Chicoric acid itself has been reported to inhibit HIV integrase (Charvat et al., 2006) and to exhibit antioxidant activities (Dalby-Brown et al., 2005). As dietary supplements, *Echinacea* herbal extracts have been very popular (US annual sales estimated at $100–200 million during the years 2000–2006; Tilburt, Emanuel, & Miller, 2008) but, for US consumers, basil represents a more readily available and inexpensive source of these compounds.

* Corresponding author. Tel.: +1 208 722 6701x282; fax: +1 208 722 8166.
E-mail addresses: jungmin.lee@ars.usda.gov (J. Lee), carolyn.scagel@ars.usda.gov (C.F. Scagel).

0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2008.12.075

Phenolic production in a plant can be affected by biotic and abiotic factors (Hussain et al., 2008; Lee & Martin, 2009; Toussaint et al., 2007; Yao, Zhu, & Zeng, 2007). Colonization by arbuscular mycorrhizal fungi (AMF) is known to improve plant nutrient uptake and use (Clark & Zeto, 2000), and stress tolerance (Smith & Read, 1997). AMF colonization can also alter or enhance phenolic production within the host plant (Toussaint et al., 2007; Ganz, Kailis, & Abbott, 2002). The relationship between AMF colonization and the phenolic compounds produced in plants is still not well understood (Toussaint, 2007; Yao et al., 2007).

The objectives of this study were to better identify the phenolic compounds found in the aerial portions of the fresh basil plant, and to determine the impact of AMF on plant phenolics within two cultivars. The polyphenolic contents of basil samples were determined using a blanched methanol extraction procedure, followed by HPLC/DAD analysis. The characterization of the polyphenolics found in the basil extracts was performed by HPLC/DAD/ESI–MS/MS.

2. Materials and methods

2.1. Plant materials

Common sweet basil and Thai basil (*Ocimum basilicum*, specific cultivar names unknown) were purchased from a local market in Nampa, ID, USA. Edible portions (mainly leaves) were separated from stems, and both fractions were then stored frozen at −80 °C prior to extraction. Purchased sweet basil will be referred to as sweet basil, for clarification hereafter, to distinguish it from the other (sweet) basil samples of the AMF portion of this study. Two basil cultivars (Genovese Italian and Purple Petra) were used to examine the effect of inoculation with AMF on plant phenolic production.

Echinacea whole plant herbal supplement extract was purchased from a local shop (Nampa, ID, USA) and analyzed by HPLC. The *Echinacea* herbal extract contained *E. purpurea*, according to the manufacturer's label. All chemicals for polyphenolic extraction and HPLC analysis were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless indicated otherwise. Solvents and chemicals for this investigation were of analytical and high performance liquid chromatography (HPLC) grade. Purified chicoric acid standard was purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ, USA).

2.2. AMF inoculum

The AMF, *Glomus intraradices*, originally obtained from Native Plants Incorporated (Salt Lake City, UT, USA), was propagated in pot cultures on roots of bunching onion (*Allium cepa* L. 'White Lisbon') grown in a 1:1 mixture of Willamette Valley alluvial silt loam and river sand (8 mg kg^{-1} available phosphorus, pH 6.3) for five months. Inoculum consisted of a mixture of the soil substrate, extraradical hyphae and spores, and colonized root segments (<2 mm in length). Propagule numbers (10 propagules g^{-1} of soil substrate) in the inoculum used in this study were estimated using the MPN (most probable number) method (Woomer, 1994).

2.3. Plant culture and AMF inoculation

Surface-sterilized (30% sodium hypochlorite for 10 min, followed by a sterile water rinse) basil seeds were sown in 4 in. pots (Gage Dura Pot #GDP400) containing a steam-pasteurized (60 °C for 30 min) 3:1 mixture of Willamette Valley alluvial silt loam and vermiculite (Horticultural Vermiculite, SunGro Horticulture, Bellevue, WA, USA). Available P in the growing substrate was 3 mg kg^{-1}, and pH was 6.2. One-half of the plants were inoculated with AMF by hand-mixing 52 g of the AMF inoculum into the growing substrate in each pot before sowing seeds (AMF treatment). The same quantity of sterile (121 °C, 15 min) inoculum was mixed into the growing substrate for the remainder of the plants before sowing seeds (control). Containers were placed in a glass house and watered as needed. Supplemental light (~720 μmol PAR m^{-2} s^{-1}) was provided for 16 h d^{-1} by high-pressure multi-vapour lamps. Day/night temperatures were controlled at ~24/15 °C during the experiment. After cotyledons had fully expanded, plants were fertilized weekly with 50 ml of a liquid fertilizer (Peters Professional, Scotts Company, Maysville, OH, USA) containing 380 mg N l^{-1}, 150 mg P l^{-1}, 380 mg P l^{-1}, 64 mg S l^{-1}, 100 mg Ca l^{-1}, 36 mg Mg l^{-1}, 4.6 mg Fe l^{-1}, 18 mg Cl l^{-1}, 0.55 mg Mn l^{-1}, 0.37 mg B l^{-1}, 0.024 mg Zn l^{-1}, 0.06 mg Cu l^{-1}, and 0.006 mg Mo l^{-1}. Plants were pruned back, to three nodes, six weeks after germination and flowers that began to develop nine weeks after germination were removed from plants.

2.4. Plant harvest and colonization assessment

All plants were destructively harvested 16 weeks after germination and separated into leaves, stems, and roots. Substrate was removed from roots by washing and samples of roots were taken for assessing AMF colonization. Samples of leaves and stems were taken for phenolic analyses (described below). The samples of fresh roots were cleared and stained, using a modified procedure of Phillips and Hayman (1970), in which lacto-phenol was replaced with lacto-glycerin, and assessed for AMF colonization. AMF colonization was measured on ~1 cm sections of root samples, using the Biermann and Linderman (1980) method.

2.5. Sample preparation and extraction for polyphenolic analysis

Frozen samples were liquid nitrogen-powdered and extracted with acidified methanol (0.1% formic acid, v/v) as described in Lee and Finn (2007) with the following modification: An IKA M20 Universal mill (IKA works Inc., Wilmington, NC, USA) was used to grind frozen samples. Frozen powder (5 g) with the first methanol addition was blanched in a boiling water bath for 5 min, then immediately chilled in an ice bath for 10 min. This chilled mixture was centrifuged and the pellet was re-extracted, two additional times, with acidified methanol, as described in Lee and Finn (2007). Methanol was evaporated with a RapidVap Vacuum Evaporation System (Labconco Corp., Kansas City, MO, USA) at 40 °C, and re-dissolved in water to a final volume of 25 ml. Aqueous extracts were stored at −80 °C prior to further analyses.

To examine the importance of inactivating native enzymes in phenolic retention, sets of sweet basil leaves were powdered and then extracted, with and without the initial blanching step (in triplicates).

2.6. Total anthocyanins (TACY) and total phenolics (TP) determination

TACY were analyzed as described in Lee, Durst, and Wrolstad (2005) and TP were determined as described by Waterhouse (2002). TACY absorbance was measured at 520 and 700 nm, and expressed as mg cyanidin-3-glucoside 100 g^{-1} tissue. TP absorbance was taken at 765 nm and was expressed as mg gallic acid 100 g^{-1} tissue. A SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA) was used. Both measurements were conducted in duplicate.

2.7. HPLC/DAD and HPLC/DAD/ESI–MS/MS analyses

Basil and *Echinacea* herbal extracts were analyzed as described in Lee and Finn (2007). Briefly, solid phase C18 (Sep-Pak Plus;

Waters Corp. Milford, MA, USA) clean-up, prior to HPLC analysis, was conducted (Lee & Finn, 2007). HPLC/DAD (HP1100; Agilent Technologies, Inc., Palo Alto, CA, USA) and HPLC/DAD/ESI–MS/MS (MS was an XCT ion trap mass spectrometer, Agilent Technologies, Inc.) system and conditions were performed, using a previously published method (Lee & Finn, 2007). ESI was operated in negative ionization mode. Phenolic acids were quantified as caffeic acid at 320 nm. Flavonol-glycoside was quantified as quercetin-rutinoside at 370 nm. Both classes of phenolics were quantified, based on external standards. Phenolic compounds were identified, based on UV–visible (UV–vis) spectra, retention time, molecular ions and fragmentation ions. When available, authentic standards were used to aid the identification of phenolics (namely, caffeic acid, chicoric acid, quercetin-rutinoside, and rosmarinic acid). A sum of the individual phenolics (determined by HPLC) will be referred to as total polyphenolics, to distinguish these values from TP in this paper. Phenolics other than anthocyanins will be referred to as polyphenolics in the following sections for conciseness.

2.8. Statistical analysis

Statistica for Windows version 7.1 (StatSoft Inc., Tulsa, OK, USA) was used for *t*-test calculations and one-way analysis of variance (ANOVA) for all measurements ($\alpha = 0.05$). The Pearson product moment correlation coefficient (r) was used to assess the relationship between the TP obtained via spectrophotometer and total polyphenolics by HPLC ($\alpha = 0.05$).

3. Results and discussion

3.1. Identification and quantification of phenolics from basil

Three major phenolic compounds were found in purchased fresh sweet and Thai basil leaf samples (Tables 1–3), and in samples of 'Genovese Italian' and 'Purple Petra' (Table 4). Most of the basil phenolics were derivatives of caffeic acid. The dominant phenolic in all samples, regardless of plant tissue or cultivar (Tables 2–4), was rosmarinic acid (peak 11; Table 1 and Fig. 1A). Rosmarinic acid (caffeic acid dimer) has been found as the dominant phenolic in basil by numerous other researchers (Javanmardi et al., 2002; Jayasinghe et al., 2003; Nguyen & Niemeyer, 2008). Rosmarinic acid is also the main phenolic acid in many other herbs of the family *Lamiaceae*, namely, oregano, sage, thyme, rosemary, spearmint, and marjoram (Fecka & Turek, 2008; Kivilompolo & Hyotylainen, 2007).

The second most dominant phenolic, and previously unidentified in basil leaves, was chicoric acid (peak 7; Table 1 and Fig. 1A). Identity of chicoric acid in basil leaves (Fig. 1A) was confirmed by comparison with a purchased pure standard (Fig. 1C) and *E. purpurea* herbal extracts (Fig. 1B), by evaluating retention time, UV–vis spectra (Fig. 2), and MS information (Table 1). The UV–vis spectra of chicoric acid from the three samples (sweet basil leaves, *E. purpurea* herbal extract, and pure standard) are overlaid in Fig. 2. Chicoric acid was present in all basil leaves examined (Tables 2 and 4), with Thai basil leaf samples having the highest level of chicoric acid (88.5 mg 100 g^{-1} fresh weight; Table 2). Making direct comparisons between chicoric acid concentrations in basil leaves to concentrations reported in *E. purpurea* is complicated by other findings being expressed as mg g^{-1} of dry weight or ml of extract (Bergeron, Gafner, Batcha, & Angerhofer, 2002; Molgaard et al., 2003; Perry et al., 2001). A small level of chicoric acid was found in Thai basil stems (Table 2). Chicoric acid was not detected in stems of sweet basil, 'Genovese Italian', or 'Purple Petra'.

The third major phenolic identified was caftaric acid (caffeoyltartaric acid). This is the first report identifying caftaric acid in basil leaves, though tartaric acid has been reported (Leal et al., 2008). Identification of caftaric acid (peak 2 in Table 1 and Fig. 1A) was confirmed by comparing retention time, UV–vis spectra, molecular ion, and fragmented ions to caftaric acid from 'Pinot noir' grapes (Lee & Martin, 2009). The basil caftaric acid peak was also compared to the caftaric acid peak of commercially-available *E. purpurea* herbal extracts (Fig. 1B), since they have been reported to contain caftaric acid (Bergeron et al., 2002; Perry et al., 2001). Caftaric acid was not detected in any stem samples from basil (Tables 2 and 4).

We suspect that the potency of fraction VI from basil leaves, described by Jayasinghe et al. (2003), as having the most antioxidant activity, was due to chicoric acid. Though unidentified, Jayasinghe et al. (2003) suspected the dominant peak in fraction VI to be a caffeoyl derivative. Results presented by Nguyen and Niemeyer (2008) showed two other major 330 nm absorbers (present in their basil samples with much higher peak areas than caffeic acid), which the authors did not identify. We suspect that these unidentified peaks were caftaric acid and chicoric acid.

Jayasinghe et al. (2003) reported the presence of numerous phenolic compounds in basil leaves (~20 compounds). We did not find similar variation in phenolic composition in our basil leaf samples, possibly due to differences in cultivars, plant-growing conditions, sample preparation methods and analytical conditions. Our data show some variation in phenolic composition between the basil cultivars. Peaks 1, 4, 6, and 9 were present in extracts from sweet basil leaves but not Thai basil leaf samples (Table 2). Similarly, peaks 2, 4, 6, 8, and 10 were present in extracts from leaves of 'Genovese Italian' but not 'Purple Petra'. Additionally, the

Table 1
Phenolics found in market-purchased basil leaves (*Ocimum basilicum*) from an unnamed cultivar of sweet basil. Of the basil cultivars examined in this study, this cultivar contained the most diverse assortment of compounds. ESI was operated in negative mode.

Peaks[a]	Compounds	Molecular ions $[M-H]^-$	Fragmented ions (m/z)	λ_{max} (nm)[b]
1	Caffeic acid derivative	359	197, 179 [caffeic acid-H]$^-$	335
2	Caftaric acid	311	179 [caffeic acid-H]-, 149 [tartaric acid-H]$^-$	330, 300s
3	Cinnamyl malic acid	295	163 [M-H-malic acid]$^-$ = [coumaric acid-H]$^-$, 131 [malic acid-H]$^-$, 113, 119	315, 295s
4	Feruloyl tartaric acid	324	282, 193 [ferulic acid-H]$^-$, 149 [tartaric acid-H]$^-$	330, 300s
5	Caffeic acid	179	135	320, 290s
6	Caffeic acid derivative	179	149, 135	330, 300s
7	Chicoric acid	472	309 [M-H-tartaric acid]$^-$, 291 [M-H-caffeic acid]$^-$, 179 [caffeic acid-H]$^-$	330, 300s
8	Lithospermic acid	536	491	330, 300s
9	Quercetin-rutinoside	609	301 [M-H-rutinoside]$^-$ = [quercetin-H]$^-$	360
10	Cinnamic acid derivative	359	161	335, 285s
11	Rosmarinic acid	359	161	330, 290s

[a] Peaks listed in the order of elution. Peak 2 was identified with the aid of 'Pinot noir' grape extracts (Lee & Martin, 2009). Peaks 5, 7, 9, and 11 were co-chromatographed with purchased standards. All other peaks (1, 3, 4, 6, 8, and 10) were tentatively identified.
[b] s: shoulder. Peaks 1–8 and 10–11 were quantified as caffeic acid. Peak 9 was quantified as quercetin-rutinoside.

Table 2
Phenolic composition of the market-purchased basil (*Ocimum basilicum*) leaves and stems from two unnamed cultivars of sweet and Thai basil.

Components[a]	Sweet basil				Thai basil			
	Leaves		Stems		Leaves		Stems	
TACY	nd[b]		nd		2	(0)	13	(0)
TP	523	(15)[c]	244	(4)	605	(6)	231	(1)
Polyphenolics								
Peak 2: caftaric acid	16.5	(1.18)	nd		1.93	(0.08)	nd	
Peak 7: chicoric acid	51.8	(0.65)	nd		88.5	(2.03)	0.30	(0.04)
Peak 11: rosmarinic acid	112	(1.86)	31.9	(4.74)	128	(1.50)	40.3	(1.05)
Other minor compounds	28.3	(0.23)	3.98	(0.77)	17.6	(1.44)	9.41	(0.06)
Total polyphenolics	208	(3.50)	35.9	(5.51)	236	(2.41)	50.0	(1.10)

[a] Samples were split into leaf and stem fractions before extraction and chemical analyses. All values expressed on a fresh weight basis. TACY: total anthocyanins (mg of cyanidin-glucoside · 100 g^{-1} tissue). TP: total phenolics (mg gallic acid · 100 g^{-1} tissue). Polyphenolics: polyphenolic compounds as determined by HPLC (mg · 100 g^{-1} of tissue). Other minor compounds: sum of peaks 1, 3, 4, 5, 6, 8, 9, and 10 (sweet basil leaves); 5, 9, and 10 (sweet basil stems); 3, 5, 8, and 10 (Thai basil leaves); 3, 5, 9, and 10 (Thai basil stems). Total polyphenolics: phenolics other than anthocyanins.
[b] nd: not detected.
[c] Means ($n = 3$) followed by standard errors in parentheses.

Table 3
Comparison of phenolic composition of market-purchased basil (*Ocimum basilicum*) leaves from an unnamed sweet basil cultivar after extraction with (blanched) and without (non-blanched) an initial blanching step before phenolic analyses by spectrophotometer and HPLC.

Component[a]	Blanched extracts	Non-blanched extracts
TP	523a (15)[b]	478b (1)
Polyphenolics		
Peak 2: caftaric acid	16.5a (1.18)	14.6a (2.45)
Peak 7: chicoric acid	51.8a (0.65)	46.9b (1.34)
Peak 11: rosmarinic acid	112a (1.86)	89.6b (1.71)
Other minor compounds	28.3a (0.23)	24.6b (0.68)
Total polyphenolics	208a (3.50)	176b (5.78)

[a] All values expressed on a fresh weight basis. TP: total phenolics (mg gallic acid · 100 g^{-1} tissue). Polyphenolics: phenolic compounds, as determined by HPLC (mg · 100 g^{-1} of tissue). Other minor compounds: sum of peaks 1, 3, 4, 5, 6, 8, 9, and 10. Total polyphenolics: phenolics other than anthocyanins.
[b] Means ($n = 3$) followed by standard errors in parentheses. Means followed by different lower-case letters within a row are significantly different ($p \leqslant 0.05$, *t*-test).

Jayasinghe et al. (2003) results were based on extractions from dried basil leaves, compared to fresh basil samples analyzed in our study. As fresh leaves contain more water, concentrations of phenolics would be lower when expressed on a fresh weight basis, and a decrease in concentration may have contributed towards fewer compounds being identified in our study.

E. purpurea has been reported to be a major human dietary source of chicoric acid (Nusslein, Kurzmann, Bauer, & Kreis, 2000), but our data from basil and the reports of chicoric acid in iceberg lettuce (*Lactuca sativa* L.; Baur, Klaiber, Koblo, & Carle, 2004) and chicory (*Cichorium intybus* L.- endives, radicchio, Innocenti et al., 2005) indicate that this is clearly not the case. Iceberg lettuce and chicory are within the *Asteraceae*, and basil is among the *Lamiaceae*. Olah, Radu, Mogosan, Hanganu, and Gocan (2003) found chicoric acid in a herb, known as Cat's whiskers (*Orthosiphon aristatus*) in the *Lamiaceae*. Chicoric acid might be present in other food sources, but has so far been unidentified due to its rapid degradation by native enzymes, and the difficulty in obtaining a purified standard for comparison (Nusslein et al., 2000; Perry et al., 2001). Identification of chicoric acid in other food sources may be important because it has been reported to have stronger antioxidant activity than has rosmarinic acid (Dalby-Brown et al., 2005; Jayasinghe et al., 2003).

Several minor peaks were identified from leaf and stem samples (Table 1 and Fig. 1A). All minor peaks were at concentrations of less than 6.6 mg 100 g^{-1}. Quercetin-rutinoside was a minor peak of the sweet basil leaf and stem samples, which had been reported in *O. basilicum* (Grayer et al., 2002) and other *Ocimum* species by Grayer, Kite, Abou-Zaid, and Archer (2000), Grayer et al. (2002). Lithospermic acid (a caffeic acid trimer) was detected in all basil leaf samples, but not in stems, though it has been found in basil roots (Tada et al.,

Table 4
Summary of phenolic concentration and composition in leaves and stems of two basil cultivars (*Ocimum basilicum* 'Genovese Italian' and 'Purple Petra') inoculated (AMF) or not (control) with the arbuscular mycorrhizal fungus, *Glomus intraradices*.

Component[a]	'Genovese Italian'				'Purple Petra'			
	Control		AMF		Control		AMF	
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
TACY	nd[b]	nd	nd	nd	65a (2)	41a (5)	88b (7)	48a (5)
TP	644a[c] (79)	376a (48)	489a (63)	335a (31)	325a (21)	172a (12)	390a (27)	190a (25)
Polyphenolics								
Peak 2: caftaric acid	3.34a (1.30)	nd	1.94a (0.45)	nd	0.44a (0.08)	nd	0.36a (0.11)	nd
Peak 7: chicoric acid	24.8a (3.63)	nd	19.4a (1.84)	nd	11.42a (1.49)	nd	10.9a (1.99)	nd
Peak 11: rosmarinic acid	117a (19.4)	72.4a (9.25)	80.5a (8.78)	60.3a (2.56)	35.2a (2.93)	29.2a (3.86)	39.4a (3.36)	36.6a (5.32)
Other minor compounds	14.7a (2.77)	4.47a (0.19)	10.7a (0.88)	4.33a (0.64)	3.46a (0.42)	2.66a (0.43)	3.23a (0.19)	2.96a (0.14)
Total polyphenolics	160a (25.4)	76.8a (9.24)	112a (11.7)	64.6a (2.42)	50.5a (4.38)	31.9a (3.90)	53.9a (5.12)	39.6a (5.31)
Fresh Weight (g per plant)	20.2a (2.7)	16.6a (1.8)	15.7a (1.1)	15.4a (0.4)	22.9a (2.7)	9.8a (0.6)	21.8a (1.1)	9.5a (1.4)

[a] All phenolic concentrations are expressed on a fresh weight basis. TACY: total anthocyanins (mg of cyanidin-glucoside · 100 g^{-1} tissue). TP: total phenolics (mg gallic acid · 100 g^{-1} tissue). Polyphenolics: polyphenolic compounds, as determined by HPLC/DAD (mg · 100 g^{-1} of tissue). Other minor compounds: sum of peaks 3, 4, 5, 6, 8, and 10 ('Genovese Italian' leaves); 5 and 10 ('Genovese Italian' stems); 5 and 9 ('Purple Petra' leaves); 5 and 10 ('Purple Petra' stems). Total polyphenolics: phenolics other than anthocyanins determined by HPLC.
[b] nd: not detected.
[c] Means ($n = 4$) followed by standard errors in parentheses. Means within a cultivar and tissue followed by different lower-case letters within a row are significantly different ($p \leqslant 0.05$, one-way ANOVA).

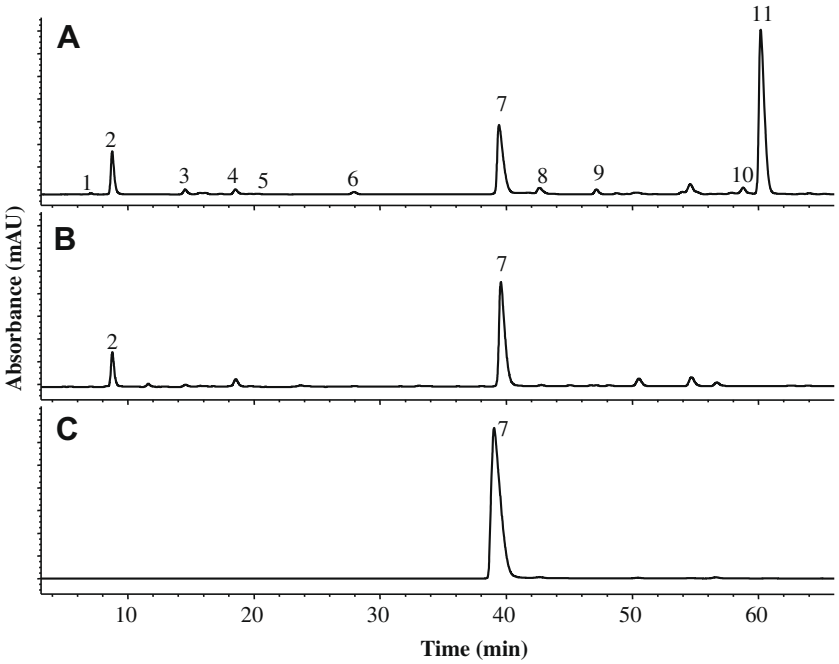

Fig. 1. Chromatogram of market-purchased basil (*Ocimum basilicum*) leaf extract from an unnamed sweet basil cultivar (A), commercially-available *Echinacea purpurea* herbal extract (B), and chicoric acid standard (C) monitored at 320 nm. Peak assignments are listed in Table 1. All other peaks were monitored and quantified at 320 nm and 370 nm.

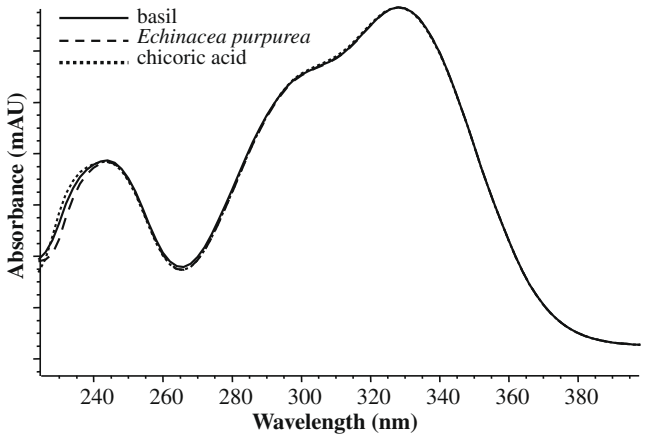

Fig. 2. Superimposed UV–vis spectra chicoric acid peaks from market-purchased basil (*Ocimum basilicum*) leaves from an unnamed sweet basil cultivar, commercially-available *Echinacea purpurea* extract, and a pure chicoric acid standard.

1996). 5-Caffeoylquinic acid has been reported in basil (Jayasinghe et al., 2003), but 5-caffeoylquinic acid and its isomers (isomerized as described by Nagels, Van Dongen, DeBrucker, & DePooter, 1980 that produced 1-caffeoylquinic acid, 3-caffeolylquinic acid, and 4-caffeoylquinic acid) were not found in these samples. Chromatograms extracted from a total ion current (TIC) MS scan chromatogram, in the negative ion mode at m/z 353, revealed weak chromatographic peaks, but they were not at the corresponding retention times of the caffeoylqunic acid isomers (Lee & Finn, 2007; Nagels et al., 1980). Other cinnamic acid derivatives were also present in minor quantities (Table 1 and Fig. 1A). Peaks other than caftaric, chicoric, and rosmarinic acids were combined and listed as other minor compounds (Tables 2–4). All basil stem fractions had lower phenolic concentrations and less variation in phenolic composition than did the corresponding cultivar's leaf sample.

No anthocyanins were detected in sweet basil leaves or stems (Table 2). Thai basil stems had higher concentrations of TACY than leaves, as expected from visual observations (Thai basil leaves had no visual purple colour and the petiole had a slight purple hue) (Table 2). Thai basil leaves had higher levels of TP than sweet basil leaves. The purchased basil stems had similar levels of TP.

Some studies (Nguyen & Niemeyer, 2008; Toussaint et al., 2007) have only reported the concentration of caffeic acid and rosmarinic acid in basil leaves, leading to a potential underestimation of basil phenolic content by HPLC. The results from our studies indicate that, if only caffeic and rosmarinic acids were measured, approximately 25–50% of the other individual phenolics in leaves and 4–18% of those in stems, would not be accounted for, based on mg 100 g^{-1} of fresh weight.

3.2. Effect of blanching prior to chemical extraction of sweet basil leaves

During preliminary research (data not shown), we observed browning of basil tissue during sample preparation for phenolic analyses, similar to that described by others (Dogan, Turan, Dogan, Arslan, & Alkan, 2005); therefore we decided to evaluate the effect of an initial blanching step prior to chemical extraction of basil tissue. The TP value for blanched extracts was 9.0% higher than that of non-blanched extracts (Table 3). Blanched extracts had higher levels of chicoric acid (9.6%), rosmarinic acid (19.9%), other minor compounds (13.1%) and total polyphenolics by HPLC (15.7%) than did non-blanched extracts. Although not statistically significant, caftaric acid was higher in blanched extracts, as well. These observed differences between blanched and non-blanched extracts might be greater in studies utilizing procedures with extended extraction times that do not include liquid nitrogen powdering or organic solvent. Extended extraction time, as well as higher temperatures, favours native enzymatic degradation of phenolics and could contribute toward large differences in phenolic concentrations. Perry et al. (2001) showed a greater than 50% loss in phenolics when *Echinacea* was initially extracted with water only, which is more favourable for native enzyme activity than is organic solvent extraction.

An initial blanching step, prior to extraction or processing, has often been included with samples known to turn brown rapidly, e.g. potatoes (Rodriguez-Saona, Giusti, & Wrolstad, 1998). Blanching has also been used to denature (deactivate) native enzymes (i.e. polyphenoloxidase) in basil (Dogan et al., 2005) and blueberries (Lee, Durst, & Wrolstad, 2002). In our study, it is possible that blanching facilitated phenolic extraction compared to non-blanched samples. Nusslein et al. (2000) and Dogan et al. (2005) demonstrated how chicoric acid (*E. purpurea*) and rosmarinic acid (basil) rapidly degrade in the presence of active native enzymes in analytical preparations. Susceptibility of basil phenolics to oxidation can be minimized, using an initial blanching step to maximize phenolic extraction and retention prior to analysis.

3.3. Influence of analysis method on TP values

The TP and total polyphenolic values we obtained from basil, using different methods (spectrophotometer vs. HPLC), were significantly correlated (market-purchased basil: $r = 0.991$, $p \leqslant 0.05$; AMF-study basil: $r = 0.934$, $p \leqslant 0.05$). TP values from spectrophotometric analyses tended to be higher than the total polyphenolic values determined by HPLC/DAD, as previously observed (Lee & Finn, 2007; Lee & Martin, 2009). Higher TP values from spectrophotometric analysis are partially due to the interference of compounds found in plant samples (Waterhouse, 2002).

3.4. Effects of AMF on phenolic composition of basil

Plants inoculated with AMF had high (>67%) root colonization and non-inoculated plants showed no sign of AMF colonization. Inoculation of basil with AMF had no effect on fresh weight of leaves or stems (Table 4), or dry weight of leaves, stems, or roots (data not shown). Leaves of 'Purple Petra' plants inoculated with AMF had significantly higher TACY than had non-inoculated plants, although the same trend was observed in 'Purple Petra' stems, this difference was not statistically significant (Table 4). Inoculation of basil with AMF had no influence on TP, individual polyphenolic compounds, or total polyphenolics (Table 4).

Copetta, Lingua, and Berta (2006) tested three AMFs (*G. mosseae* BEG 12, *Gigaspora margarita* BEG 34, and *Gi. rosea* BEG 9) and demonstrated AMF altered essential oil composition of 'Genovese' basil, depending on the AMF isolated used for inoculation. The authors suggested that the increase in essential oil production in *Gi. rosea* inoculated plants may have been linked to higher plant biomass (Copetta et al., 2006). In our study, the isolate of AMF and phosphorus (P) concentration in the fertilizer used in this study were selected to grow plants with similar fresh weights, thus eliminating the effects of differences in composition due to biomass. Therefore increased TACY between inoculated and non-inoculated 'Purple Petra' plants were a consequence of inoculation.

Mycorrhizal fungi are well known for improving plant P uptake (Smith & Read, 1997). One symptom of plants under P-stress is the accumulation of anthocyanins in the leaves; therefore, there is a higher probability that non-mycorrhizal plants would have higher anthocyanin concentrations than would mycorrhizal plants. Toler, Morton, and Cumming (2005), however, reported that mycorrhizal sorghum had higher concentrations of anthocyanins than had non-mycorrhizal plants, but only when plants were under stress from elevated concentrations of copper (Cu) in the growing substrate. Several authors have speculated that many of the physiological advantages of mycorrhiza are only measurable when plants are growing under physiologically stressful conditions (Smith & Read, 1997). In our study, inoculated and non-inoculated plants contained similar amounts of nutrients (data not shown) and were provided with similar amounts of water. Therefore, increased TACY in inoculated 'Purple Petra' plants were probably not a result of increased water or nutrient stress.

Using the same AMF species, Toussaint et al. (2007) reported that inoculation of 'Genova' basil resulted in no significant differences in concentrations of rosmarinic acid or caffeic acid in shoots (combination of stems and leaves), compared to non-inoculated plants when oven-dried samples from 7-week-old plants were analyzed using HPLC. Interestingly, they also reported variation in responses of basil to different isolates of AMF. Plants inoculated with *G. caledonium* or *G. mosseae* had higher concentrations of caffeic acid in shoots than had non-inoculated plants, and the response was independent of the effects of AMF on plant P nutrition. In our study, lack of response in basil phenolics to AMF inoculation may be a result of the isolate of AMF used in the study and its compatibility with the cultivars of basil.

Ganz et al. (2002) found that AMF (mixture of *G. invermaium*, *Acaulospora laevis*, and *Scutellospora calospora*; strains not reported) inoculated olive plants had lower phenolic content than had non-AMF-inoculated plants, in both leaves and roots. Basil root phenolic composition was not the focus of this study. In the future, it would be interesting to determine whether root phenolics were altered due to AMF colonization, as reported by Toussaint et al. (2007) for rosmarinic acid and caffeic acid when basil plants were inoculated with specific AMF isolates.

The effects of AMF inoculation on variation in basil phenolics due to cultivar, AMF isolate and colonization, and plant age will be explored in the future, as interest in this research has increased and there have been few studies in this field (Toussaint et al., 2007). Plant secondary metabolites actively participate in plant-microbe interactions, such as those in AMF-plant symbioses (Zhi-Lin, Chuan-Chao, & Lian-Quing, 2007). Enhancement of secondary metabolite accumulation in plants is of great importance in production of culinary herbs with antioxidant activity. The relationship between AMF and phenolic content alteration, within various plants, has been reviewed by Yao et al. (2007) and Toussaint (2007) and both agree that numerous factors need clarification in order to better define the relationship between AMF and its host plant's secondary metabolite production.

4. Conclusions

To the best of our knowledge, this is the first paper to identify the presence of chicoric acid and caftaric acid in basil. The presence of chicoric acid has now been reported in plants of the family *Asteraceae* (*Echinacea*, iceberg lettuce, and chicory) and *Lamiaceae* (basil and Cat's whiskers). This is the first report of chicoric acid in basil, and only the second to find chicoric acid in plants of the *Lamiaceae* family.

The response of basil phenolics to inoculation with AMF appears to depend on cultivar, polyphenolic class, plant tissue, and may also depend on AMF isolate.

Regarding the potential health benefits of agricultural foodstuffs, this study provides relevant information related to the dietary availability of chicoric acid. Chicoric acid is much more readily available from common and inexpensive basil leaves than from *E. purpurea* extracts, which was traditionally considered to be the main source of chicoric acid.

Acknowledgements

We thank Chris Rennaker, Jesse Mitchell, Anne Davis, and Rose Jepson of the USDA-ARS for technical assistance. This work was funded by USDA-ARS CRIS numbers 5358-21000-041-00D and 5358-12210-003-00D.

References

Barnes, J., Anderson, L. A., Gibbons, S., & Philipson, J. D. (2005). *Echinacea* species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): A review of their chemistry, pharmacology and clinical properties. *Journal of Pharmacy and Pharmacology, 57*, 929–954.

Baur, S., Klaiber, R. G., Koblo, A., & Carle, R. (2004). Effect of different washing procedures on phenolic metabolism of shredded, packaged iceberg lettuce during storage. *Journal of Agricultural and Food Chemistry, 52*, 7017–7025.

Bergeron, C., Gafner, S., Batcha, L. L., & Angerhofer, C. K. (2002). Stabilization of caffeic acid derivatives in *Echinacea purpurea* L. glycerin extract. *Journal of Agricultural and Food Chemistry, 50*, 3967–3970.

Biermann, B. J., & Linderman, R. G. (1980). Quantifying vesicular-arbuscular mycorrhizae: A proposed method towards standardization. *New Phytologist, 87*, 63–67.

Charvat, T. T., Lee, D. J., Robinson, W. E., & Chamberlin, A. R. (2006). Design, synthesis, and biological evaluation of chicoric acid analogs as inhibitors of HIV-a integrase. *Bioorganic and Medicinal Chemistry, 14*, 4552–4567.

Clark, R. B., & Zeto, S. K. (2000). Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition, 23*, 867–902.

Copetta, A., Lingua, G., & Berta, G. (2006). Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. *Genovese*. *Mycorrhiza, 16*, 485–494.

Dalby-Brown, L., Barsett, H., Landbo, A. R., Meyer, A. S., & Molgaard, P. (2005). Synergistic antioxidative effects of alkamides, caffeic acid derivatives, and polysaccharide fractions from *Echinacea purpurea* on in vitro oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry, 53*, 9413–9423.

Dogan, S., Turan, P., Dogan, M., Arslan, O., & Alkan, M. (2005). Purification and characterization of *Ocimum basilicum* L. polyphenol oxidase. *Journal of Agricultural and Food Chemistry, 53*, 10224–10230.

Fecka, I., & Turek, S. (2008). Determination of polyphenolic compounds in commercial herbal drugs and spices from *Lamiaceae*: Thyme, wild thyme and sweet marjoram by chromatographic techniques. *Food Chemistry, 108*, 1039–1053.

Ganz, T. R., Kailis, S. G., & Abbott, L. K. (2002). Mycorrhizal colonization and its effect on growth phosphorus uptake and tissue phenolic content in the European olive (*Olea europaea* L.). *Advances in Horticultural Science, 16*, 109–116.

Grayer, R. J., Kite, G. C., Abou-Zaid, M., & Archer, L. J. (2000). The application of atmospheric pressure chemical ionization liquid chromatography–mass spectrometery in the chemotaxonomic study of flavonoids: Characterization of flavonoids from *Ocimum gratissimum* var. gratissimum. *Phytochemical Analysis, 11*, 257–267.

Grayer, R. J., Kite, G. C., Veitch, N. C., Eckert, M. R., Marin, P. D., Senanayake, P., et al. (2002). Leaf flavonoid glycosides as chemosystematic characters in *Ocimum*. *Biochemical Systematics and Ecology, 30*, 327–342.

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology, 124*, 91–97.

Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry, 108*, 986–995.

Innocenti, M., Gallori, S., Giaccherini, C., Ieri, F., Vincieri, F. F., & Mulinacci, N. (2005). Evaluation of the phenolic content in the aerial parts of different varieties of *Cichorium intybus* L.. *Journal of Agricultural and Food Chemistry, 53*, 6497–6502.

Javanmardi, J., Khalighi, A., Kashi, A., Bais, H. P., & Vivanco, J. M. (2002). Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. *Journal of Agricultural and Food Chemistry, 50*, 5878–5883.

Jayasinghe, C., Gotoh, N., Aoki, T., & Wada, S. (2003). Phenolics comparison and antioxidant activity of sweet basil (*Ocimum basilicum* L.). *Journal of Agricultural and Food Chemistry, 51*, 4442–4449.

Kivilompolo, M., & Hyotylainen, T. (2007). Comprehensive two-dimensional liquid chromatography in analysis of *Lamiaceae* herbs: Characterization and quantification of antioxidant phenolic acids. *Journal of Chromatography A, 1145*, 155–164.

Kosar, M., Dorman, H. J. D., & Hiltunen, R. (2005). Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected *Lamiaceae* species. *Food Chemistry, 91*, 525–533.

Leal, P. F., Maia, N. B., Caemello, Q. A. C., Catharino, R. R., Eberlin, M. N., & Meireles, M. A. (2008). Sweet basil (*Ocimum basilicum*) extracts obtained by supercritical fluid extraction (SFE): Global yields, chemical composition, antioxidant activity, and estimation of the cost of manufacturing. *Food and Bioprocess Technology, 1*, 326–338.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2002). Impact of juice processing on blueberry anthocyanins and polyphenolics: Comparison of two pretreatments. *Journal of Food Science, 67*, 1660–1667.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International, 88*, 1269–1278.

Lee, J., & Finn, C. E. (2007). Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. *Journal of the Science of Food and Agriculture, 87*, 2665–2675.

Lee, J., & Martin, R. R. (2009). Influence of grapevine leafroll associated viruses (GLRaV-2 and -3) on the fruit composition of Oregon *Vitis vinifera* L. Cv. Pinot noir: Phenolics. *Food Chemistry, 112*, 889–896.

Makri, O., & Kintzios, S. (2008). *Ocimum* sp. (basil): Botany, cultivation, pharmaceutical properties, and biotechnology. *Journal of Herbs Spices and Medicinal Plants, 13*, 123–150.

Molgaard, P., Johnsen, S., Christensen, P., & Cornett, C. (2003). HPLC method validated for the simultaneous analysis of cichoric acid and alkamides in *Echinacea purpurea* plants and products. *Journal of Agricultural and Food Chemistry, 51*, 6922–6933.

Nagels, L., Van Dongen, W., DeBrucker, J., & DePooter, H. (1980). High performance liquid chromatographic separation of naturally occurring esters of phenolic acids. *Journal of Chromatography, 187*, 181–187.

Nguyen, P. M., & Niemeyer, E. D. (2008). Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). *Journal of Agricultural and Food Chemistry, 56*, 8685–8691.

Nusslein, B., Kurzmann, M., Bauer, R., & Kreis, W. (2000). Enzymatic degradation of chicoric acid in *Echinacea purpurea* preparations. *Journal of Natural Products, 63*, 1615–1618.

Olah, N., Radu, L., Mogosan, C., Hanganu, D., & Gocan, S. (2003). Phytochemical and pharmacological studies on *Orthosiphon stamineus* Benth. (*Lamiaceae*) hydroalcoholic extracts. *Journal of Pharmaceutical and Biomedical Analysis, 33*, 117–123.

Perry, N. B., Burgess, E. J., & Glennie, V. I. (2001). *Echinacea* standardization: Analytical methods for phenolic compounds and typical levels in medicinal species. *Journal of the Science of Food and Agriculture, 49*, 1702–1706.

Phillips, J. M., & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society, 55*, 158–161.

Rodriguez-Saona, L., Giusti, M. M., & Wrolstad, R. E. (1998). Anthocyanin pigment composition of red-fleshed potatoes. *Journal of Food Science, 63*, 458–465.

Smith, S. E., & Read, D. J. (1997). *Mycorrhizal symbiosis* (2nd ed.). San Diego, Calif., USA: Academic Press.

Tada, H., Murakami, Y., Omoto, T., Shimomura, K., & Ishimaru, K. (1996). Rosmarinic acid and related phenolics in hairy root cultures of *Ocimum basilicum*. *Phytochemistry, 42*, 431–434.

Tilburt, J. C., Emanuel, E. J., & Miller, F. G. (2008). Does the evidence make a difference in consumer behavior? Sales of supplements before and after publication of negative research results. *Journal of General Internal Medicine, 23*, 1495–1498.

Toler, H. D., Morton, J. B., & Cumming, J. R. (2005). Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. *Water, Air, and Soil Pollution, 164*, 155–172.

Toussaint, J. P. (2007). Investigating physiological changes in the aerial parts of AM plants: What do we know and where should we be heading? *Mycorrhiza, 17*, 349–353.

Toussaint, J. P., Smith, F. A., & Smith, S. E. (2007). Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. *Mycorrhiza, 17*, 291–297.

Waterhouse, A. L. (2002). Unit I1.1: Polyphenolics: Determination of total phenolics. In R. E. Wrolstad (Ed.), *Current protocols in food analytical chemistry* (pp. 1–4). New York: John Wiley & Sons.

Woomer, P. L. (1994). Most probable number counts. In R. W. Weaver (Ed.), *Methods of soil analysis, part 2* (pp. 59–79). Madison, Wis., USA: Soil Sci. Soc. Am., Inc.,.

Yanishlieva, N. V., Marinova, E., & Pokorny, J. (2006). Natural antioxidants from herbs and spices. *European Journal of Lipid Science and Technology, 108*, 776–793.

Yao, Q., Zhu, H. H., & Zeng, R. S. (2007). Role of phenolic compounds in plant defense: Induced by arbuscular mycorrhizal fungi. *Allelopathy Journal, 20*, 1–14.

Zhi-Lin, Y., Chuan-Chao, D., & Lian-Quing, C. (2007). Regulation and accumulation of secondary metabolites in plant-fungus symbiotic system. *African Journal of Biotechnology, 6*, 1266–1271.