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Short communication

Caffeic acid derivatives in dried Lamiaceae and *Echinacea purpurea* products

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ABSTRACT

The concentrations of caffeic acid derivatives within Lamiaceae and *Echinacea* (herb, spice, tea, and dietary supplement forms) readily available in the US marketplace ($n = 72$) were determined. After the first identification of chicoric acid in *Ocimum basilicum* (basil), the extent to which chicoric acid could be found within the family Lamiaceae was investigated. The dominant phenolic acid in all Lamiaceae samples was rosmarinic acid, which ranged from 2.04 mg/100 g (one of 12 oregano samples) to 622.28 mg/100 g (lemon balm). Of the herbs tested in this study (marjoram, oregano, peppermint, rosemary, sage, spearmint, and thyme from the family Lamiaceae), only basil and lemon balm (*Melissa officinalis*) contained chicoric acid. Basil samples (starting material and resulting end product) obtained from an industry cooperator, showed substantial phenolic deficiency as a result of processing (approximately 78% loss).

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1. Introduction

Dried herbs that are often used as cooking ingredients (seasonings) possess potential health benefits due to their natural phenolics, which are not fully investigated (Yanishlieva et al., 2006; Clifford, 1999), though they are universally recognized for their flavour contributions. After our recent report (Lee and Scagel, 2010) of dried basil being deficient in phenolics compared to fresh basil, we proceeded to determine if this was the case for other dried Lamiaceae products that are easily obtained from the marketplace.

The presence of chicoric acid in aerial portions of basil plants, and in dried basil samples were first reported by Lee and Scagel (2009). Although there have been cases of chicoric acid found in plants other than *Echinacea purpurea*, historically, *E. purpurea* (from the family Asteraceae) has been viewed as the main or the only source of chicoric acid (also known as cichoric acid; dicaffeoyltartaric acid) as it is the predominant phenolic in *E. purpurea* and its resulting products (Molgaard et al., 2003). Chicoric acid is thought to act as an antioxidant, anti-inflammatory, antiviral, and immuno-stimulator (Barnes et al., 2005; and references therein). Chicoric acid has been found in iceberg lettuce (Baur et al., 2004), endives, radicchio, and chicory (Innocenti et al., 2005; Mulinacci et al., 2001), which are all from the family containing *Echinacea*: Asteraceae. Cat's whiskers (*Orthosiphon aristatus*) from the family Lamiaceae were reported to contain chicoric acid, demonstrating that plant families besides Asteraceae can contain chicoric acid, and that chicoric acid might be more ubiquitous than previously thought (Olah et al., 2003).

After we first reported the presence of chicoric acid in the leaves and stems of basil (family Lamiaceae; Lee and

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Scagel, 2009), another research group confirmed our identification (Shiga et al., 2009). We then surveyed the range of chicoric acid within readily available commercial basil products (Lee and Scagel, 2010). To our surprise, they all contained only low levels of phenolics (mainly phenolic acids and one flavonol-glycoside ranging from 42.41 to 277.10 mg/100 g as-is dried herb). At least one major herb and spice company has recognized that they could readily improve phenolic retention in their products by improving plant selection, post-harvest handling, processing methods, storage, among others (personal communication, anonymous). There have also been efforts to enhance phenolic content in the basil plant by arbuscular mycorrhizal fungi (AMF; Lee and Scagel, 2009; Nell et al., 2009) inoculation, but additional work is needed to understand how AMF or other field techniques (altered plant nutrition, etc.) can reliably increase desired phenolics.

For this current study, we wanted to explore other herb products from the Lamiaceae family for chicoric acid. Our primary objective was to identify and determine chicoric acid levels (or absence) in everyday Lamiaceae and Asteraceae that could be easily purchased from local US markets. *E. purpurea* products were included as a baseline for available chicoric acid and to determine the range of chicoric acid within market available products. The secondary objective was to determine basil phenolic retention during processing by analyzing fresh plant (starting material) and their resultant freeze–dried products, from commercially produced samples.

2. Materials and methods

2.1. Samples

Dried forms of basil, lemon balm, marjoram, mugwort, oregano, peppermint, rosemary, sage, spearmint, and thyme commonly available were purchased from local marketplaces in Boise, Nampa, and Caldwell, ID, USA, in April 2009. Samples represented 15 companies and were categorized as herb, spice, tea, or dietary supplement (all dried form only). Sample package, cost per 100 g, company name, visual color, etc. were recorded upon arrival to the laboratory. All purchased samples were within their "best by" or "expiration" date.

Details regarding genus and species of all samples are given in Table 1. Samples were coded and the abbreviations are also listed in Table 1. Samples were immediately kept at −80 °C upon arrival to the laboratory. *Echinacea* and mugwort samples were from the family Asteraceae, while the rest of the herbs, spices, and teas were in the family Lamiaceae.

A separate group of samples was obtained through an industry collaborator from their growing and production year 2009. We received four basil samples (*Ocimum basilicum* L. cv. not disclosed): fresh basil harvested from the end of the growing season and frozen prior to shipment, IQF (individually quick frozen) optimum growing-peak basil also stored frozen, blanched basil also stored frozen, and freeze–dried product stored cool and dry (four samples). Unfortunately starting material with its corresponding end product of conventionally dehydrated basil, was not accessible during the time of this study. After arrival at the laboratory, all four samples were stored at −80 °C prior to extraction and analysis.

2.2. Reagents, chemicals, and standards

All chemicals and reagents were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise specified. Solvents and chemicals used in this investigation were analytical or high performance liquid chromatography (HPLC) grade. Chicoric acid and lithospermic acid A (also known as lithospermic acid) were purchased from Cerilliant Corp. (Round Rock, TX, USA).

2.3. Extraction and HPLC analysis

All samples were liquid nitrogen powdered with a mortar and pestle as described by Lee and Scagel (2009). To avoid added handling during extraction, the samples purchased as encapsulated supplements had their gelatin capsule portions removed prior to freezing. Only pure *E. purpurea* containing capsules were obtained. Details of the extraction procedure are described by Lee and Scagel (2009, 2010). Briefly, all liquid nitrogen powdered samples were extracted with acidified methanol (0.1% formic acid in methanol; total three times). An initial boiling step was included to inhibit degradation of phenolic compounds during extraction, as described by Lee and Scagel (2009). Aqueous extracts were purified with

Table 1 – Genus and species names for samples purchased from local market. All available samples (n = 72) were purchased.

Common name	Genus and species	Number of samples
Basil	*Ocimum basilicum*	6
Echinacea purpurea	*Echinacea purpurea*	9
Lemon balm	*Melissa officinalis*	1
Marjoram	*Origanum majorana*	3
Mugwort	*Artemisia vulgaris*	1
Oregano	*Origanum vulgare*	12
Peppermint	*Mentha piperita*	4
Rosemary	*Rosmarinus officinalis*	12
Sage	*Salvia apiana*	11
Spearmint	*Mentha spicata*	3
Thyme	*Thymus vulgaris*	10

All samples belonged to the family Lamiaceae, except for *E. pupurea* and mugwort, which were in Asteraceae family.

a mini-C18 column (Sep-Pak Plus; Waters Corp., Milford, MA, USA; Lee and Finn, 2007) prior to HPLC injection. HPLC mobile phase conditions, peak detection, identification, and quantification were conducted as described in Lee and Scagel (2009, 2010) and Lee and Finn (2007), with no alterations. An Agilent HP1100 system (HPLC-DAD; Agilent Technologies Inc., Palo Alto, CA, USA) was used for the analysis. Peaks were monitored at 280, 320, and 370 nm. Identification was based on UV–VIS spectra, retention times, and mass spectra as reported by Lee and Scagel (2009, 2010), and quantified by DAD using caffeic acid or quercetin-rutinoside (rutin) as appropriate. All analyses were conducted in duplicate.

Pearson product moment correlation coefficient (r) was used to describe price of the samples per 100 g, versus total phenolic acid comparison using Statistica for Windows version 7.2 (StatSoft Inc., Tulsa, OK, USA). Differences among the component results obtained from industry basil processing samples were tested using one-way analysis of variances (ANOVA) and the Tukey HSD (Honest Significant Difference; $\alpha = 0.05$).

3. Results and discussion

3.1. *Commercial Lamiaceae and Asteraceae phenolics*

The contents of caffeic acid derivative (phenolic acid) in all samples are summarized in Table 2. All values were reported as mg of caffeic acid/100 g as-is (the form consumers would obtain). Dried basil samples were the only ones that contained all four caffeic acid derivatives (caftaric acid, caffeic acid, chicoric acid, and rosmarinic acid in the order of HPLC elution), as previously reported (Lee and Scagel, 2009, 2010). A representative chromatogram of basil sample from this HPLC method can be seen in Lee and Scagel (2009). Caftaric acid, chicoric acid, and rosmarinic acid structures can be found in Lee and Scagel (2010). Rosmarinic acid was the main caffeic acid derivative found in dried basil. *E. purpurea* samples contained caftaric acid, caffeic acid, and chicoric acid (main compound), as previously reported (Lee and Scagel, 2009, 2010). Lemon balm was uniquely high in rosmarinic acid and was the only sample besides basil or *E. purpurea* to contain chicoric acid. Rosmarinic acid was the dominant caffeic acid derivative in marjoram, mugwort, oregano, peppermint, rosemary, sage, spearmint, and thyme, as reported by many others (Wang et al., 2004; Areias et al., 2000; Fecka and Turek, 2008; Yanishlieva et al., 2006). Caffeic acid was the other phenolic acid found in marjoram, mugwort, oregano, peppermint, rosemary, sage, spearmint, and thyme.

Of the herbs examined, basil samples contained the least amount of rosmarinic acid (Table 2), and mugwort the second least. This study's basil samples were lower in rosmarinic acid and chicoric acid than basil samples we previously tested, where the dried samples contained 11.43–166.83 mg and 6.48–16.24 mg/100 g of these caffeic acid derivatives, respectively (Lee and Scagel, 2010). The levels of chicoric acid in *Echinacea* samples were higher than basil or lemon balm, but varied greatly (68.88 and 242.50 mg/100 g, Lee and Scagel, 2010), as noticed in earlier studies (Lee and Scagel, 2010; Molgaard et al., 2003). Only spearmint samples did not vary widely in their rosmarinic acid content (Table 2), while oregano samples were the most extreme, ranging from 2.04 to 575.80 mg/100 g. Two oregano samples out of 12 were extremely low in rosmarinic acid content, 2.04 and 3.12 mg/100 g, respectively.

From these findings, chicoric acid may not be ubiquitous within the family Lamiaceae, and might be limited to basil, lemon balm (both this study), and cat's whiskers (Olah et al., 2003). Phenolic acid contents of these samples ($n = 72$) were not correlated with the sample price (US$/100 g; $r = 0.093$, $p > 0.05$). High priced herb items were low in their phenolic acid content, which might be due to contemporary industry standards for herbs and spices that determine quality not by retention of phenolics, but by a minimum essential oil content (personal communication, anonymous).

One *Echinacea* capsule product stood out visually while removing its gelatin capsules, it was dark brown while the other *Echinacea* products were light yellow to green. This brown sample had the lowest chicoric acid content (42.37 mg/100 g) of the *Echinacea* samples. It would be interesting if darkness or brown colour could be accurately used as a quality indicator of *E. purpurea* capsules. However, that was not within the scope of this study, though rapid colour change

Table 2 – Content of caffeic acid derivatives in dried Lamiaceae and Asteraceae products (mg of caffeic acid/100 g as-is).

Common name	n	Caftaric acid	Caffeic acid	Chicoric acid	Rosmarinic acid
Basil	6	1.05[a] (0.18) *0.47–1.75*[b]	5.44 (1.34) *2.81–10.98*	17.50 (1.94) *11.70–25.67*	40.63 (13.01) *14.59–86.01*
Echinacea purpurea	9	5.19 (1.64) *1.40–17.03*	11.59 (6.57) *1.05–62.84*	108.71 (22.20) *42.37–258.70*	n.p.[c]
Lemon balm	1	n.d.[d]	2.68	2.23	622.28
Marjoram	3	n.d.	6.70 (0.70) *5.71–8.05*	n.d.	270.14 (72.34) *165.55–409.01*
Mugwort	1	n.d.	2.54	n.d.	96.97
Oregano	12	n.d.	4.14 (0.36) *2.73–7.53*	n.d.	212.29 (44.28) *2.04–575.80*
Peppermint	4	n.d.	5.76 (1.07) *3.70–8.01*	n.d.	248.73 (76.05) *66.47–406.69*
Rosemary	12	n.d.	4.36 (0.29) *2.58–5.61*	n.d.	265.46 (16.91) *187.36–370.22*
Sage	11	n.d.	13.06 (1.02) *7.99–19.75*	n.d.	222.81 (16.50) *121.41–316.72*
Spearmint	3	n.d.	4.80 (1.85) *2.29–8.41*	n.d.	242.55 (9.08) *225.96–257.24*
Thyme	10	n.d.	6.94 (0.76) *2.09–10.55*	n.d.	244.58 (24.94) *131.71–357.02*

a Mean values followed by a standard error value in parenthesis.
b Values in italic are ranges obtained from the different samples.
c n.p., not present.
d n.d., not detected.

(i.e., browning) has been noted in studies exploring methods to improve *E. purpurea* processing for better phenolic retention (Molgaard et al., 2003; Nusslein et al., 2000). A close scrutiny of the combined data from this study and our previous chicoric acid work, the *Echinacea* samples (42.37–258.70 mg/100 g; $n = 11$; Lee and Scagel, 2010) were higher in their chicoric acid content than the basil and lemon balm samples (2.23–25.67 mg/100 g; $n = 15$). Again, *Echinacea* samples were found to have a wide range of chicoric acid concentration (Lee and Scagel, 2010; Molgaard et al., 2003).

3.2. *Phenolic retention of four samples of basil from industry production*

As reported previously (Lee and Scagel, 2009; 2010), 11 polyphenolic compounds were identified in these commercially produced samples. Beyond rosmarinic acid (dominant phenolic found in these samples), chicoric acid, caffeic acid, and caftaric acid, and all other seven peaks were summed together as 'other peaks' (see Fig. 1). Peak identities are reported by Lee and Scagel (2009, 2010), with a description of how these peaks were quantified and identified. Of these samples, freeze–dried basil contained the highest concentration of polyphenolics (422.92 mg/100 g as-is) due to its decreased mass due to water removal. IQF growing-peak basil (and stored at −23 °C) was the lowest in polyphenolics (126.78 mg/100 g as-is) when compared to the other three samples. Blanched basil samples (199.32 mg/100 g as-is) and end of growing season plants (170.55 mg/100 g as-is) maintained their polyphenolic levels better, but it is well established that blanching improves polyphenolic retention by inhibiting native enzyme activity (Lee and Scagel, 2009; Lee et al., 2002; Wills and Stuart, 2000). Blanching and frozen storage, freezing and frozen storage, and freeze-drying likely altered the proportion of the polyphenolics found. Notably, rosmarinic acid was significantly lower in IQF optimum growing-peak basil.

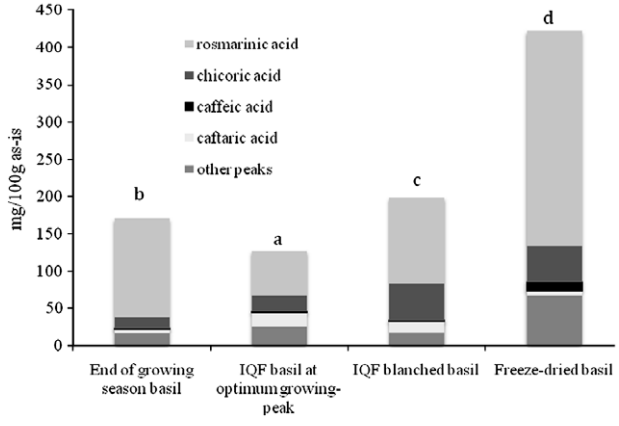

Fig. 1 – Phenolics of industry obtained basil samples from 2009 production and processing. Content reported as mg of caffeic acid /100 g as-is. 'Other peaks' values were the summation of seven minor peaks as reported in Lee and Scagel (2009, 2010). IQF stands for individually quick frozen. Different lowercase letter indicates significant difference (Tukey's HSD).

Using the accompanying product datasheet obtained from the company to account for moisture content, the corresponding polyphenolic levels from highest to lowest would have been: blanched basil (2491.51 mg/100 g dry weight), end of growing season basil (2131.87 mg/100 g dry weight), IQF optimum growing-peak basil (1584.80 mg/100 g dry weight), and lastly freeze–dried basil (445.18 mg/100 g dry weight). Blanching basil prior to freeze-drying could aid in the retention of phenolics. Since the polyphenolics in basil are particularly susceptible to degradation (Lee and Scagel, 2009), it is not surprising that the freeze–dried basil lost approximately 78% of its polyphenolics during the process. A more comprehensive assessment would entail taking fresh basil plants, processing them (via air drying, freeze-drying, blanching before drying, etc.), and subjecting the dehydrated samples to controlled transportation or storage abuse. Phenolic loss has been observed in plant dehydration, although simple practices like blanching, cultivar selection, and other variables have demonstrated improved retention of phenolics (Lee and Scagel, 2009; Lee et al., 2002; Wills and Stuart, 2000; Di Cesare et al., 2003; Tanko et al., 2005).

4. Conclusion

This is the first report to identify chicoric acid in lemon balm. Of the nine herbs from Lamiaceae tested here, only basil and lemon balm samples contained chicoric acid. All Lamiaceae samples examined in this study contained rosmarinic acid. The herb samples readily available from the local market ranged greatly in their phenolic acid content, but it appeared that implementing existing techniques could improve their retention of phytochemicals. Herbs have the ability to make food taste better, but careful cultivar selection, processing improvements, and better storage practices could mitigate the loss of their phenolics, volatile compounds, and chlorophylls, from field to fork. That would perhaps give these seasonings even a better flavour and value.

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