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Phylloquinone content of herbs, spices and seasonings

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ABSTRACT

Culinary herbs and spices contain a variety of bioactive compounds including phylloquinone, the most common dietary form of vitamin K. In this study, we analyzed the phylloquinone content of a large number of commonly available culinary herbs, spices, and seasonings. Samples of fresh herbs ($n = 19$), dried herbs ($n = 28$), spices and seeds ($n = 26$), seasoning blends ($n = 9$), and other flavor enhancers ($n = 11$) were purchased in Montreal (Quebec, Canada) and Washington (DC, USA). All samples were analyzed in quadruplicate using standardized HPLC procedures. Most fresh herbs contained between 450 and 1200 μg of phylloquinone/100 g. Dried herbs were even richer with some (cilantro, marjoram, parsley) showing concentrations of ≈3000 μg/100 g. Phylloquinone content of spices and seeds was relatively low (5–250 μg/100 g), while being highly variable among seasoning blends (2.3–1878 μg/100 g). According to our results, portions of only 3 g of herbs can increase daily intakes of phylloquinone by up to 100 μg, contributing significantly to the daily vitamin K intake. Herbs can thus be important food sources of phylloquinone and should be accounted for when assessing vitamin K intakes in research or in patients treated with vitamin K antagonists. Future research should focus on the bioavailability of phylloquinone in these products.

1. Introduction

The use of spices in the United States (US) has constantly increased over the last 45 years, growing from 1.2 pounds per capita in 1966 to 3.5 pounds per capita in 2010 (United States Department of Agriculture Economic Research Service). The US immigration, the growing popularity of ethnic foods, and the need to enhance the flavor of low-salt/low-fat foods are thought to explain this increase (Buzzanell et al., 1995). In culinary art, herbs and spices are mainly used to flavor foods and infuse vegetable oils and vinegars. In dietetic practice, they are often presented as an alternative to salt in the prevention and management of hypertension. In research, herbs and spices are gaining in interest because they contain a variety of bioactive compounds (*e.g.* polyphenols, menthol, retinol, carotenoids, curcumin) known for their anti-microbial, anti-oxidant, anti-cancer, and anti-inflammatory health benefits (Jungbauer and Medjakovic, 2012; Kaefer and Milner, 2008; Viuda-Martos et al., 2011).

One bioactive component of herbs and spices is phylloquinone. Phylloquinone is the primary form of vitamin K in the North American diet. It is synthesized by green plants as an electron acceptor in the photosystem I. As a result, green leafy vegetables (*e.g.* spinach, kale) contribute the most to phylloquinone intakes; other contributors include other plant foods such as some vegetable oils, root/stem vegetables, and fruits (*e.g.* kiwifruit, grapes, avocado) (Booth et al., 1996; Duggan et al., 2004; Fenton et al., 1997; Thane et al., 2002, 2006). Interestingly, the highest concentrations of phylloquinone in foods are found in culinary herbs. For example, phylloquinone contents of dried basil and dried parsley, as reported in the US Department of Agriculture (USDA) nutrient database (NDB), are 1714.5 μg/100 g and 1359.5 μg/100 g, respectively (U.S. Department of Agriculture, 2014). In comparison, raw spinach is three times less concentrated *i.e.* 482.9 μg/100 g. Some spices, such as chilli powder and grounded cloves, can also have relatively high phylloquinone content (>100 μg/100 g).

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Despite the fact that herbs are likely the richest food sources of phylloquinone, published studies do not provide data on their relative contribution to phylloquinone intakes. This may be in part explained by the challenge of quantitatively assessing herb and spice consumption, as they are mostly consumed in small amounts and in conjunction with other foods (Kaefer and Milner, 2008). Another explanation lies in the fact that phylloquinone content values of many herbs and spices commonly used in diet (*e.g.* bay leaves, mint, rosemary, allspice) are missing from nutrient databases (U.S. Department of Agriculture, 2014).

In the present study, we analyzed the phylloquinone content of a large number of commonly available fresh and dried herbs, spices and seeds, seasoning blends, and other flavor enhancers, many of which having missing values in nutrient databases. Also, because infusing vegetable oils and vinegar with herbs is a growing culinary art practice, we examined whether infusing olive oil and white vinegar with basil could increase their phylloquinone content. Practical implications of our results are discussed.

2. Materials and methods

Samples of fresh herbs (n = 19), dried herbs (n = 28), spices and seeds (n = 26), seasoning blends (n = 9), and other flavor enhancers (n = 11) were purchased in supermarkets from Montreal (Quebec, Canada) and Washington (DC, USA). All samples were protected from light from the moment of purchase in order to minimize light degradation of phylloquinone. To guarantee freshness, fresh herbs were purchased less than 24 h before phylloquinone analysis and stored at 4 °C. For each category of dried herb and spice, as many as 3 samples were purchased and kept at room temperature until analysis.

Two cups of olive oil and two cups of white vinegar were infused at 4 °C with one cup of fresh basil for one week. Three infusions were prepared: olive oil infused with basil and protected from light, olive oil infused with basil without light protection, and white vinegar infused with basil without light protection. Samples of non-infused olive oil and white vinegar were used as controls.

2.1. Phylloquinone content analysis

The phylloquinone content of herbs, spices, seasonings, and herb-infused oil and vinegar was determined in quadruplicate by reverse-phase high-performance chromatography (HPLC) using post-column reduction and fluorescence detection (Hitachi, FL detector, L-7480) as previously described (Ferland and Sadowski, 1992a,b). All samples were analyzed under yellow light, in the laboratory of Dr. G. Ferland.

Edible portions of dried and fresh herbs, spices and seasonings were initially pulverized using a mill grinder or mortar and pestle, depending on the matrix, to obtain a homogeneous sample. After pulverization, samples were weighted (0.05–0.20 g) and transferred to a 50 mL polypropylene tube to which was added 3 mL of H_2O and 6 mL of isopropanol/hexane mixture (3:2, v/v). An appropriate amount of internal standard containing 1–1000 ng/50 μL of dihydrovitamin K1 (K_1H_2) was then added, depending on the final dilution. The mixture was then dispersed by sonication for 2 min, transferred to a glass tube, and centrifuged at 3000 rpm for 10 min at 4 °C until phase separation. For analyses of oils and vinegars, samples were weighted (0.25–1.0 g), transferred to a glass tube with an appropriate amount of internal standard (K_1H_2), and extracted with hexane by shaking vigorously during 3 min.

After phase separation, the upper hexane layer was transferred to a glass tube and evaporated to dryness at 45 °C under nitrogen (N_2). The residue was dissolved in hexane and an aliquot of the appropriate dilution was loaded into a preconditioned 3 mL solid-phase extraction silica column (J.T. Baker Inc., Phillisburg, NJ). The loaded column was then washed with 8 mL of hexane, and phylloquinone was eluted with 8 mL of 3% diethyl ether in hexane (97:3, v/v). The eluate was dried at 45 °C under N_2 and then dissolved in 0.02 mL of 100% methylene chloride and 0.18 mL of methanol containing aqueous phase (10 mM zinc chloride, 5 mM acetic acid and 5 mM sodium acetate). The final mixture (200 μL) was injected into the chromatography system (Waters Limited, Missisauga, ON, Canada) and run through a C18 column (3 μm ODS-Hypersil column, 150 mm × 4.6 mm i.d., Thermo Scientific Inc, PA), with as isocratic mobile phase consisting of 94.5% methanol, 5% methylene chloride and 0.5% aqueous phase. The phylloquinone content of samples was quantified according to the internal standard method based on peak areas and expressed as μg/100 g of food weight.

2.2. Data analysis

Mean phylloquinone content of herbs and spices are presented per 100 g. Variability between quadruplicates is expressed as CV (%). Data from each brand of dried herbs and spices are presented separately. When possible, the entry reference number of the corresponding product from the Nutrient Database (NDB) of the USDA, Release 27, is specified in the tables, as well as its specific phylloquinone content value when available.

3. Results and discussion

3.1. Phylloquinone content of herbs, spices, and seasonings

The present study confirmed that culinary herbs and some spices are among the richest food sources of phylloquinone. Indeed, most fresh herbs contained 450–1200 μg per 100 g (Table 1). Dried herbs were even richer with some, such as cilantro, marjoram, and parsley, showing values near 3000 μg/100 g (Table 2). Except for allspice that contained an appreciable amount of phylloquinone, spices have relatively low phylloquinone contents (5–250 μg/100 g), most likely because they are from parts of the plant that have low-photosynthetic activity (*e.g.* buds, seeds, bark, berries, roots). Phylloquinone content of seasoning blends are very variable but some, such as the Italian and poultry seasonings, showed really high phylloquinone content (>1000 μg/100 g). To our knowledge, this is the first report providing a comprehensive assessment of phylloquinone content values of a large number of commonly used fresh and dried herbs, spices and seeds, seasoning blends, and other flavor enhancers.

Comparison of our dataset with that of the USDA NDB is limited as many products from the latter were missing (n = 25), or did not have phylloquinone content value (n = 19). However, where comparisons are possible, phylloquinone values from our samples are generally higher and in some cases, differences are large (*e.g.* dried marjoram, dried parsley) (Tables 1 and 2). Discrepancies could be attributable to numerous factors including cultivar, leaf canopy position, plant maturation, growing geographical location and/or conditions (soil, climate) (Ferland and Sadowski, 1992b; Lester et al., 2010, 2013). Herbs and spices processing and storage (*e.g.* drying process, light exposure, shelf time before purchasing) could also influence phylloquinone contents (Ferland and Sadowski, 1992a; Lester et al., 2010). In the present study, the phylloquinone content of fresh mint purchased on three different occasions during summer was found to increase from July through September, suggesting that months of purchase may also represent a source of variation (Table 1). One noticeable difference among fresh herbs is our phylloquinone value for fresh parsley that is much lower than that of the USDA NDB (640–787 μg/100 g *vs.* 1640 μg/100 g). Two studies from UK and Finland have