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Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes

Ulla Justesen *, Pia Knuthsen

Institute of Food Research and Nutrition, Danish Veterinary and Food Administration, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

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Abstract

Many herbs are known as excellent sources of natural antioxidants, and consumption of fresh herbs in the diet may therefore contribute to the daily antioxidant intake. The present study was performed in order to quantify flavonoids in commonly eaten fresh herbs. Fifteen fresh herbs (basil, chives, coriander, cress, dill, lemon balm, lovage, oregano, parsley, rosemary, sage, spearmint, tarragon, thyme, and watercress) were analysed by HPLC and mass spectrometry. Five major flavonoid aglycones were detected and quantified by HPLC after acid hydrolysis: apigenin, isorhamnetin, kaempferol, luteolin, and quercetin. The highest levels of flavonoids were found in parsley (510–630 mg apigenin /100 g), lovage (170 mg quercetin/100g), mint (18–100 mg apigenin/100 g), and dill (48–110 mg quercetin/100 g). Mass spectrometric detection, using atmospheric pressure chemical ionisation (APCI), was used to verify the presence of flavonoids in the hydrolysed extracts of herbs. Some traditional Danish dishes contain herbs, particularly parsley, dill, cress and chives, and the contribution to the flavonoid intake by consumption of these dishes was calculated.

Keywords: Fresh herbs; Flavonoid intake; Danish dishes

1. Introduction

Phenolic compounds constitute a major class of secondary plant metabolites. They include phenolic acids and flavonoids, and particularly the latter is a highly diverse subgroup. The phenolics are present in fruits, vegetables and other plant products consumed in a normal diet. It is well established that diets rich in fruits and vegetables can inhibit the development of major diseases such as CHD and cancers (Block, Patterson, & Subar, 1992; Ness & Fowles, 1997). The beneficial effects are partly being ascribed to the natural antioxidants in foods (Vinson et al., 1999). Flavonoids may be stronger antioxidants than antioxidant vitamins. It is still being discussed, however, whether flavonoids exert such effects in vivo (Duthie, 1999). In Ayurveda, Indian medicine, various spices and herbs are described to show medicinal effects (Srivastava & Mustafa, 1993).

We have previously screened fruits, vegetables and beverages for the contents of flavanones, flavones and flavonols, and quantified seven major flavonoids as aglycones (Justesen, Knuthsen & Leth, 1998). The presence of flavonoids and other phenolic antioxidants has been reported in a number of herbs (Justesen, 2000), e.g. in basil (Baritaux, Amiot, Richard & Nicolas, 1991; Grayer, Bryan, Veitch, Goldstone, Paton & Wollenweber, 1996), chives (Trichopoulou et al., 2000), coriander (Kunzemannn & Herrmann, 1976), dill (Teuber & Herrmann, 1978), oregano (El-Ansari, El-Negoumy & El-Desoky, 1996; Kanazawa, Kawasaki, Samejima, Ashida & Danno, 1995), parsley (Justesen et al., 1998; Tomas, Mataix & Carpena, 1972), rosemary (Cuvelier, Richard & Berset, 1996, Maillard, Giampaoli, & Cuvelier, 1996; Okamura, Haraguchi, Hashimoto & Yagi, 1994), sage (Cuvelier et al., 1996; Maillard et al., 1996; Samejima, Kanazawa, Ashida & Danno, 1995), mint (Samejima et al., 1995; Yamamura, Ozawa, Ohtani, Kasai & Yamasaki, 1998), tarragon (Hoffmann & Hermann, 1982) and thyme (Guillén & Manzanos, 1998; Haraguchi, Saito, Ishikawa, Kataoka, Tamura & Mizutani, 1996; Morimitsu, Yoshida, Esaki & Hirota,

* Corresponding author. Carlsberg Research Center, Gl. Carlsberg Vej 10, DK-2500 Valby Copenhagen, Denmark. Tel.: +44-3327-5280; fax: +44-3327-4764.

E-mail address: ulj@crc.dk (U. Justesen).

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1995; Samejima, Kanazawa, Ashida & Danno, 1995). The majority of flavones, flavonols, and flavanones were present as glucuronides or glycosides and were in most of the studies determined as aglycones.

The average daily intake of flavonols and flavanones in Denmark has been calculated by us (Justesen, Knuthsen, Andersen & Leth, 2000). The study did not include the contributions made by consumption of fresh herbs, as it is negligible on an average daily basis.

The present study was performed in order to describe flavonoid intake from consumption of herbs in traditional Danish dishes, as these may provide a considerable contribution to the daily flavonoid intake on the occasions when fresh herbs are used in cooking.

2. Materials and methods

The following fresh herbs were purchased at a local grocery store, freeze-dried and kept at −20°C until use: basil (*Ocimum basilicum*), chives (*Allium schoenoprasum*), coriander (*Coriandrum sativum*), cress (*Lepidium sativum*), dill (*Anethum graveolens*), lemon balm (*Melissa officinalis*), lovage (*Levisticum officinale*), mint (*Mentha var.*), oregano (*Origanum vulgare*), parsley (*Petroselinum crispum*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), tarragon (*Artemisia dranunculus*), thyme (*Thymus vulgaris*) and watercress (*Nasturtium officinale*).

2.1. Chemicals

Flavonoid standards: apigenin (4′,5,7-trihydroxyflavone, MW 270), hesperetin (3′,5,7-trihydroxy-4′-methoxyflavanone, MW 302), isorhamnetin (3,4′,5,7-tetrahydroxy-3′-methoxyflavone, MW 316), kaempferol (3,4′,5,7-tetrahydroxyflavone, MW 286), luteolin (3′,4′,5,7-tetrahydroxyflavone, MW 286), and quercetin (3,3′,4′,5,7-pentahydroxyflavone, MW 302), were purchased from Apin Chemicals (Oxon, UK) and Sigma (St. Louis, MO, USA). The standards were dissolved in DMSO to a concentration of approximately 0.1 g/l and kept at −20°C for up to 3 months.

Working solutions were made up each day by diluting 0.500 ml standard stock solution with 10 ml 62.5% aqueous methanol containing BHA (2 g/l), and 2.5 ml 6 M HCL. Solvents were supplied by Rathburn (Walkerburn, UK).

2.2. Analytical procedure

The procedure for quantitation of the flavones, flavonols, and flavanones has previously been described (Justesen et al., 1998). In brief, 0.5 g of the freeze-dried material was used for acid hydrolysis of the flavonoid glycosides. The samples were boiled for 2 h with 1.2 M HCl in 50% methanol, and filtered before HPLC analysis. Samples that were not fully hydrolysed with standard conditions (chives, dill and parsley), were boiled for 4 h with 2 M HCl in 50% methanol.

The HPLC system consisted of a Waters system (Milford, MA, USA) 717 autoinjector, 616 pump, and 996 PDA detector. The samples were analysed using a Phenomenex Prodigy (Torrance, CA, USA) RP C_{18} column (250×4.6 mm, 5 μm) protected by a guard column (LC_{18}). The mobile phase consisted of methanol–water (30:70, v/v) with 1% formic acid (A) and 100% methanol (B). The gradient was 25–86% B in 50 min at a flow-rate of 1 ml/min. UV spectra were recorded from 220 to 450 nm. Peak areas at 289, 350 or 368 nm were determined, and two standard mixtures analysed before and after the samples, were used as external standards for quantitation. Duplicate analyses were performed on different days.

Mass Spectrometry was performed on a QLC triple quadrupole (Micromass, Cheshire, UK) using the APCI inlet. Spectra were acquired in the negative ion mode, providing deprotonated molecules. For detailed description of the mass spectrometry parameters, see Justesen (2000). The mass spectrometer was connected to the UV cell outlet of the HPLC, using PEEK tubing.

3. Results and discussion

3.1. Quantitation of flavones, flavonols, and flavanones in hydrolysed samples

The contents of flavonoids in the herb samples were measured as aglycones after acid hydrolysis as described by (Hertog, Hollman & Venema, 1992; Justesen et al., 1998). Apigenin, hesperetin, isorhamnetin, kaempferol, luteolin, and quercetin were found to be most abundant, and the results are presented in Table 1. Quercetin and kaempferol were the most widespread flavonoids, as they were present in half of the herb samples included in the study. The highest levels of flavonoids were detected in parsley, mint, lovage and dill. The data presented in Table 1 are to be considered indicative of flavonoid contents in the herbs, as variety, growing conditions, country of origin, and season of harvest are expected to be sources of large variations in flavonoid contents. Trichopoulou et al. (2000) report the contents of flavonoids in chives to be 10.4 mg/100 g of quercetin, 12.5 mg/100 g of kaempferol and 8.5 mg/100 g of isorhamnetin, respectively. The chives analysed in their study were obtained from a wild growing variety, harvested on different locations on Crete. We found similar amounts of kaempferol, but lower amounts of quercetin and isorhamnetin, which could be explained by the different varieties and growing conditions of the chives analysed in the two studies.

Methoxylated flavonoids, in addition to the six flavones, flavonols and flavanones listed in Table 1, were detected as glycosides, but not quantified as they are not included in the standard method (Justesen et al., 1998).

Identification of the flavonoid glycosides is presented elsewhere (Justesen, 2000).

In the present study, we observed problems with applying the standard method of hydrolysing the flavonoid glycosides in some of the herb samples. Even when using harsh hydrolysis conditions (boiling for 4 h with 2.0 M HCl), it was not possible to perform complete hydrolysis to produce all free aglycone for quantification. Instead, it appeared that the glycosides in dill and chives were converted to a conjugate with molecular weight of the aglycone plus 190. Fig. 1 presents a HPLC chromatogram of chives, where peaks 4–6 represent free quercetin, kaempferol and isorhamnetin, and peaks 1–3 represent the respective conjugates. In Fig. 2, mass spectra of the six peaks are presented. Performing MS/MS on the flavonoid conjugates with $[M-H]^- = 491$, 475 and 505, provided fragment ions corresponding to the aglycones (quercetin, kaempferol and isorhamnetin, respectively), with no specific information of the conjugate (data not shown). We decided to add the areas of the peaks corresponding to the flavonoid conjugates to the areas of the respective aglycones in the calculations of the flavonoid contents in dill and chives, as the error disregarding the glycoside peaks would be extensive. We are not aware, of other research groups using the same method (Hertog et al., 1992; Trichopoulou et al., 2000) having experienced similar problems with single food subjects.

Table 1
Flavonoid contents (mg/100 g fresh weight) in fresh herbs, measured by HPLC–DAD after acid hydrolysis of the samples

Herb name	N[a]	Quercetin	Kaempferol	Apigenin	Luteolin	Isorhamnetin	Hesperetin
Basil (*Ocimum basilicum*)	1	–	–	–	–	–	–
Chives (*Allium schoenoprasum*)	1	3	12	–	–	5	–
Coriander (*Coriandrum sativum*)	1	5	tr[b]	–	–	–	–
Cress (*Lepidium sativum*)	2	–	13	–	–	1	–
Dill (*Anethum graveolens*)	2	48–110	16–24	–	–	15–72	–
Lemon Balm (*Melissa officinalis*)	1	–	–	–	–	–	–
Lovage (*Levisticum officinale*)	1	170	7	–	–	–	–
Mint (*Mentha* var.)	2	–	–	18–99	11–42	–	tr
Oregano (*Origanum vulgare*)	2	–	–	2–4	0–3	–	–
Parsley (*Petroselinum crispum*)	2	0–1	–	510–630	0–4	–	–
Rosemary (*Rosmarinus officinalis*)	1	–	–	–	4	–	tr
Sage (*Salvia officinalis*)	1	–	–	–	–	–	–
Tarragon (*Artemisia dranunculus*)	1	10	11	–	1	5	–
Thyme (*Thymus vulgaris*)	2	–	–	5	51	–	–
Watercress (*Nasturtium officinale*)	3	4	1	–	–	–	–

[a] Number of samples analysed in duplicate
[b] tr, trace amounts.

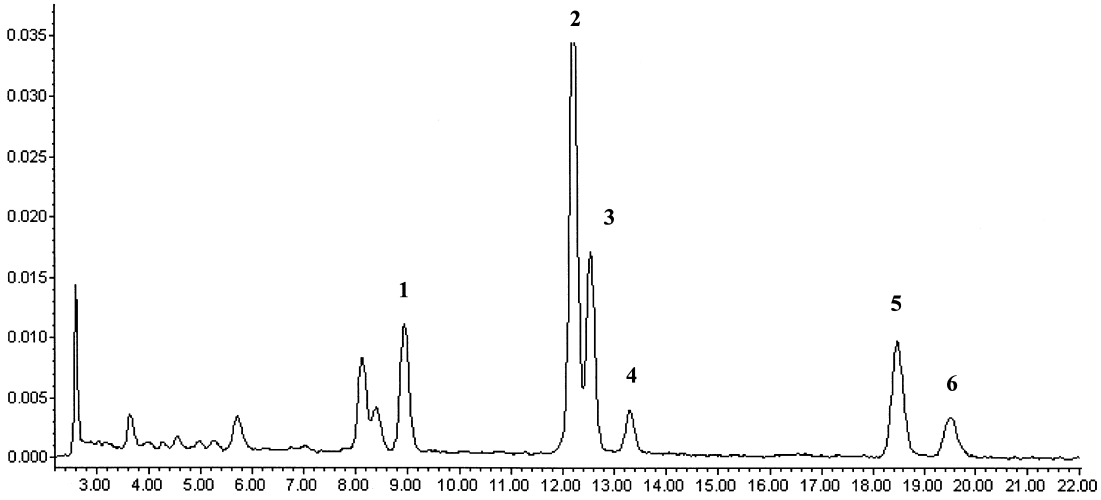

Fig. 1. HPLC–DAD chromatogram (365 nm) of chives, hydrolysed with 2 M HCl for 4 h. The six peaks correspond to flavonoid aglycones and flavonoid conjugates in the sample, as determined by LC–MS. 1. Quercetin-conjugate, 2. kaempferol-conjugate, 3. isorhamnetin-conjugate, 4. quercetin, 5. kaempferol, 6. isorhamnetin.

3.2. Flavonoid consumption

The high flavonoid contents found in some of the herbs included in this study indicate that some herbs are excellent sources of flavonoids. Consumption of a few grams of parsley, dill or spearmint would be sufficient to contribute significantly to the average daily flavonoid intake, and some herbs are consumed in quite large amounts in some dishes. In the early summer, new potatoes are traditionally served with fresh, chopped herbs, i.e. chives, cress or parsley. Other traditional Danish dishes contain herbs, mostly chives, cress, dill and parsley (Table 2). Due to the high flavonoid contents, particularly in parsley, flavonoid intake may be significantly increased by consumption of these dishes, when compared with an average daily intake at 23 mg (Hertog, Hollman, Katan & Kromhout, 1993; Justesen et al., 2000). The average daily consumption of chives, cress and dill is quite low, estimated to 0.2 g/day (Andersen et al., 1996). Herbs used less frequently in Denmark, as lovage, mint, tarragon and thyme, also contain significant amount of flavonoids. The consumption of fresh herbs is largely seasonal, belonging to the summer. Other traditional and seasonal Danish

Table 2
Traditional Danish dishes containing fresh herbs. Approximate flavonoid intake per serving is calculated

Name of dish	Major ingredients (one serving)[a]	Flavonoids per serving (mg, approx.)
Parsley Gravy[b]	2.5 g butter, 1 dl milk, 4 g wheat flour, salt, pepper 2.5–4 g fresh and chopped parsley,	Parsley 13–25 mg
New Potatoes with chives, cress or parsley[c]	250 g new, boiled potatoes, 5–7.5 g fresh, chopped chives, dill or parsley	Chives 1–2 mg Dill 4-15 mg Parsley 25–47 mg
Open Sandwich with chives, cress, dill or parsley	Tomato, cold potato, salami, rye or wheat bread, 2 g chopped chives, cress, dill or parsley	Chives < 1 mg Cress < 1 mg dill 2–4 mg Parsley 10 –13 mg
Butter with herbs (parsley or mixed herbs)[b]	13 g butter, lemon juice, 1 g parsley or mixed herbs	Parsley 5–6 mg

[a] Standards according to Andersen et al., 1996.
[b] Recipy in Andersen and Haveman, 1994
[c] Recipy in Have, 1993

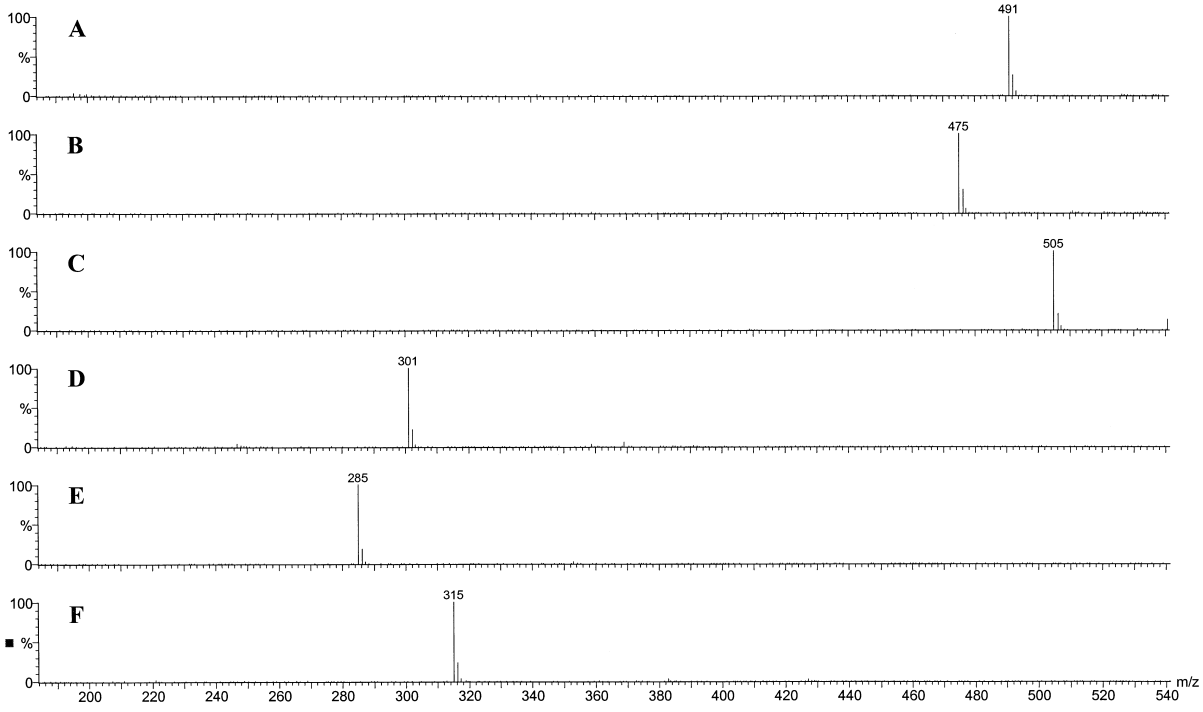

Fig. 2. Mass spectra of the six flavonoids determined in hydrolysed chives. The peak numbers refer to the peaks in the chromatogram presented in Fig. 1. (A)) Peak 1 quercetin-conjugate with $[M\text{-}H]^- = 491$, (B) peak 2 kaempferol-conjugate with $[M\text{-}H]^- = 475$, (C) peak 3 isorhamnetin-conjugate with $[M\text{-}H]^- = 505$, (D) peak 4 quercetin with $[M\text{-}H]^- = 301$, E) peak 5 kaempferol with $[M\text{-}H]^- = 285$, (F) peak 6 isorhamnetin with $[M\text{-}H]^- = 315$.

dishes are also rich in flavonoids, for example stewed kale (grønlangkål), which provide approximately 50 mg quercetin and kaempferol in total per serving (Justesen et al., 1998). This dish is typically served at Christmas and at New Years Eve, and does therefore not contribute to the calculated average flavonoid intake.

Flavonoids may only be minor antioxidant constituents in herbs, as it is known that for example rosemary has excellent antioxidant capacities due to the presence of high amounts of rosmarinic acid. Other compounds in herbs may exert adverse effects, parsley has for example been shown to induce unexpected increase in antioxidant enzymes, possibly reflecting an adaptive response by the endogenous antioxidant defence system (Nielsen et al., 1999). Parsley is known to contain fucocoumarins, natural toxicants which mainly occur in plants belonging to the families *Umbelliferae* and *Rutacae* (Søborg, Andersson & Gry, 1996). It may thus be unwise to recommend excess intake of foods with high contents of single components, before the exact biological effects are understood.

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