sci
where science meets business
www.soci.org

Analysis of furanocoumarins in vegetables (*Apiaceae*) and citrus fruits (*Rutaceae*)

Radek Peroutka, Věra Schulzová,* Petr Botek and Jana Hajšlová
Institute of Chemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic

Abstract: Several alternative approaches applicable for the analysis of furanocoumarins, toxic components occurring in some fruits and vegetables representing both *Apiaceae* and *Rutaceae* families, were tested in our study. Limits of detection (LODs) for angelicin, psoralen, bergapten, xanthotoxin, trioxsalen, isopimpinellin, sphondin, pimpinellin and isobergapten obtained by GC/MS (SIM) were in the range 0.01–0.08 μg g^{-1}. Slightly higher LODs (0.02–0.20 μg g^{-1}) were achieved by LC/MS–MS. The latter is the only alternative for analysis of bergamottin (LOD = 0.01 μg g^{-1}) in citrus fruits because this furanocoumarin is unstable under GC conditions. Regardless of the determination step used, the repeatability of the measurements (expressed as RSD) did not exceed 10%. As shown in our study the levels of furanocoumarins in celery, celeriac, parsnip, carrot, lemon and other foods obtained at a retail market varied over a wide range; the highest contents were determined in parsnip, while the levels of these toxins in carrots and citrus pulps were relatively low.
© 2007 Society of Chemical Industry

Keywords: furanocoumarins; GC/MS; LC/MS–MS; fruits; vegetables

INTRODUCTION

Furanocoumarins are toxic secondary metabolites that occur in various plant species including food crops. As shown in studies concerned with their biological effects, these compounds may demonstrate antifungal activities[1] and are phototoxic.[2,3] In the presence of long-wave UV light, furanocoumarins yield products that are able to interact with DNA, forming mono- and di-adducts. These mutagenic and carcinogenic effects were also demonstrated in animal studies.[2] It should be noted that the current toxicological database is far from complete. Therefore, in the meantime, dietary furanocoumarins should be considered as potentially harmful to consumer health. Additionally, more studies are needed to clarify the health concerns related to these phytochemicals in diet.

The highest levels of furanocoumarins in food crops[4] are typically present in plants representing the *Apiaceae* family; maximum contents (expressed as a sum of the most abundant representatives) reported in the literature were celery 45 μg g^{-1},[5] parsnip 145 μg g^{-1},[6] and parsley 112 μg g^{-1}.[7] The results were quantified on a fresh-weight basis. The content of furanocoumarins in citrus fruits (*Rutaceae* family) is rather lower, e.g. the concentration of bergamottin in grapefruit juices was only about 6 μg g^{-1}.[8] The presence of various furanocoumarins was also proved in many other plants, such as *Fabaceae, Pittosporaceae, Solanaceae, Amaranthaceae, Rosaceae, Cyperaceae* and *Moraceae*,[9] and fruit of some of these, e.g. figs, representing the last family, is also used for human consumption.

The distribution of furanocoumarins within a plant is uneven. While higher contents are typically found in leaves and other green parts, the concentrations in fruits and roots are often markedly lower. It should be noted that the levels of these plant toxins may significantly increase under stress conditions, such as attack by fungi[10] and unfavourable storage conditions resulting in damage of crop tissues.[11] Another factor to be considered when estimating the dietary intake of potential consumers is the stability of furonacoumarins while being processed under either domestic or industrial conditions. As shown in one of older studies,[12] no breakdown of furanocoumarins occurred during cooking. There is no other detailed information on this issue available in the literature.

At present, more than 50 furanocoumarins are known. Considering their chemical structure, two main subgroups can be recognised. The first involves linear furanocoumarins (e.g. psoralen, bergapten, xanthotoxin, trioxsalen, isopimpinellin, bergamottin); the second is represented by angular furanocoumarins (e.g. angelicin, pimpinellin, sphondin, isobergapten).[1]

In most published studies, isolation of furanocoumarins from plant matrices was carried out either by polar (e.g. water,[5] methanol[13,14]) or semi-polar (ethyl acetate[8,15]) solvents. Several extraction techniques were reported, utilising, for example, Soxhlet

* Correspondence to: Věra Schulzová, Institute of Chemical Technology, Department of Food Chemistry and Analysis, Technická 3, 166 28 Prague 6, Czech Republic
E-mail: vera.schulzova@vscht.cz
Contract/grant sponsor: Ministry of Education, Youth and Sports of the Czech Republic; contract/grant number: Project MSM No. 6046137305
Project COST 924
(*Received 24 July 2006; revised version received 4 January 2007; accepted 12 March 2007*)
Published online 27 June 2007; DOI: 10.1002/jsfa.2979

WILEY InterScience® DISCOVER SOMETHING GREAT

apparatus[4,16] and homogenisation with solvent using various types of grinders.[5,7,8] Supercritical fluid extraction (SFE) using supercritical carbon dioxide was also described as an isolation alternative in one paper.[4] In some studies, crude aqueous homogenate was purified by liquid–liquid extraction (toluene[5] and/or ethyl acetate[7,17] were used for partitioning) or, alternatively, by solid-phase extraction (SPE). Cartridges such as Sep-Pak silica,[4,13] extract clean SPE[5] and/or Sep-Pak C_{18},[13,14,17] have been used for this purpose. High-performance liquid chromatography (HPLC) with UV detection was the most frequently employed method[4,6–8,14,15,17,18] for the final analysis of the commonly analysed furanocoumarins. Limits of detection (LODs) of such procedures were typically higher than 20 μg kg^{-1} (fr.wt) for the particular analytes. Liquid chromatography coupled to mass spectrometry (LC/MS) has usually been the technique of choice in recent studies,[19,20] and its application also enables identification of some minor furanocoumarins. For instance, the presence of 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)-psoralen and 5,8-dimethoxy-3-(3-methyl-2,3-dihydroxybutyl)-psoralen in herbal extracts[21] was confirmed using LC/MS. Nowadays, gas chromatography coupled to mass selective detector (GC/MSD) is the most common approach for separation and quantification of thermally stable furanocoumarins[7,13,19,22] in plant extracts.

The aim of the present study was to critically assess performance characteristics of existing modern chromatographic techniques conceivable for analysis of furanocoumarins in various products. Optimised procedures were used for the examination of samples of selected vegetables and some other food commodities obtained from a retail market. In the second part of the study, the influence of storage conditions on furanocoumarin levels (related to studies[11,17,22]) in several celery cultivars was investigated.

EXPERIMENTAL

Chemicals

Angelicin, psoralen, bergapten, xanthotoxin, bergamottin and trioxsalen were purchased from Sigma–Aldrich, Steinheim, Germany; isopimpinellin was obtained from INDOFINE Chemical Company (Hillsborough, NJ, USA). The purity of all standards was 98% or greater. Ethyl acetate, purchased from Scharlau (Barcelona, Spain), was used for extraction of furanocoumarins from vegetables. Methanol, obtained from Merck (Darmstadt, Germany), was used for extraction of furanocoumarins from citrus fruits.

Materials

Samples collected within the monitoring study

Vegetables represented by parsnip (*Pastinaca sativa*), celery (*Apium graveolens* var. Dulce), celeriac (celery root, *Apium graveolens* var. Rapaceum), parsley (*Petroselinum sativum*), carrot (*Daucus carota*), citrus fruits, e.g. grapefruit (*Citrus paradisi*), lime (*Citrus arantifolia*), orange (*Citrus aurantium*), lemon (*Citrus limon*) and products made from them were purchased from a Czech retail market within the years 2003–2004.

Samples used for the experiments

Celery and celeriac cultivars used for the following experiments were obtained from the collaborating farms and the summary of the samples is shown in Table 1.

Analysis of samples

Extraction

Vegetables. All sample homogenates were prepared from washed vegetables using a Foss Tecator 2094 homogeniser (Höganäs, Sweden). Forty millilitres of ethyl acetate were added to 10 g of homogenate and the suspension was shaken (HS250 basic IKA Labortechnik, Staufen, Germany) for 30 min at the laboratory temperature, the solvent was then decanted. After filtration, extracts obtained by two repeated extractions were combined into a 100 mL volumetric flask and the volume was made up with ethyl acetate.

Dried vegetable (seasoning, soup). Six millilitres of water was added to 2 g of sample to reconstitute the original texture. After 1 h of conditioning, 40 mL of ethyl

Table 1. Summary of the samples investigated and the storage conditions applied in this study (the representative sample for analysis was prepared by homogenisation of five washed roots and/or 20 haulms representing the median size of the particular cultivar batch)

Year of farming	Vegetable	Cultivar	Part of vegetable	Storage conditions	Storage period	Experiment number
2002	Celeriac	Maxim, Radiant, Diamond, Neon	Root	Household cellar (5–13 °C)	26 weeks	1
		Maxim	Root infected by fungi (*Penicillium* sp.)	Household cellar (5–13 °C)	Identified within week 16–26	2
		Radiant	Root cut into 8 parts	Household cellar (5–13 °C)	6 weeks	3
2003	Celeriac	Albin, Kompakt, Maxim	Root, haulm	4 °C	12 weeks (4 weeks for haulm)	4
			Haulm	40 °C	4 days	
	Celery	Malachit, Avalon, Jemny	Haulm	4 °C 40 °C	4 weeks 4 days	5

DOI: 10.1002/jsfa

acetate were added to the mixture and extracted in the same way as described above for vegetables. Ten millilitres of extract was evaporated to dryness and the residue was redissolved in 1 mL of ethyl acetate.

Citrus fruits. *Pulp*: Sixty millilitres of methanol was added to 10 g of pulp homogenate (homogenisator ETA 0010, Hlinkso, Czech Republic) and suspension was mixed using a Turrax (IKA Werke, Staufen, Germany) for 10 min. This mixture was shaken for 30 min (laboratory shaker HS250 basic ICA). The extracts were filtered into a 100 mL volumetric flask and the volume was made up with methanol.

Peel: Forty millilitres of methanol was added to 10 g of homogenate (Homogenizer Foss Tecator 2094) and the suspension was shaken for 30 min (laboratory shaker HS250 basic ICA), the supernatant was decanted. After filtration, extracts obtained by two repeated extractions were combined into a 100 mL volumetric flask and the volume was made up with methanol.

Fruit tea (*dried mixture*): Forty millilitres of methanol was added to 2 g of fruit tea sample and shaken for 30 min at the laboratory temperature (laboratory shaker HS250 basic ICA). The solvent was decanted, the extraction was repeated, the combined extracts were filtered into a 100 mL volumetric flask and the volume was made up with methanol. Ten millilitres of extract were evaporated to dryness and the residue was re-dissolved in 1 mL of methanol.

Citrus juice: Forty millilitres of ethyl acetate was added to 10 g of juice and the suspension was shaken for 30 min (laboratory shaker HS250 basic ICA). The supernatant was decanted. The extracts obtained by two repeated extractions were filtered into a 100 mL volumetric flask and the volume was made up with ethyl acetate. Ten millilitres of extract were evaporated to dryness and the residue was re-dissolved in 1 mL of methanol.

Chromatographic separation

Both GC and HPLC techniques were used for separation of the sample components. In addition to a mass selective detector, UV detection was also employed for identification/quantification of the sample components.

High-performance liquid chromatography. HPLC separation was carried out using HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA). A LiChroCART column 250 × 4 mm, LiChrospher 100 RP-18, 5 mmm particles, (Merck) equipped with pre-column LiChroCART column 4 × 4 mm, LiChrospher 100 RP-18, 5 µm particles, (Merck). The column temperature was kept at 40 °C and the mobile phase flow rate was set at 1 mL min^{-1}. The following gradient was used: 0–8 min methanol/water (52:48, v/v); 8–18 min linear increase up to 100% methanol; 18–25 min 100% methanol. Prior to the injection of 20 µL of the extract, crude extracts were filtered through a 0.45 µm syringe filter (Millipore, Yonezawa, Japan).

UV detection: Furanocoumarins were detected by an HP 1100 DAD detector (Agilent Technologies, Palo Alto, CA, USA) at 248 nm. Quantification was obtained by comparing the peak areas of the target analytes with the abundances of these compounds in corresponding standards used for the calibration curve preparation.

MS detection: MS/MS measurements were carried out using LCQ Deca instrument equipped with an ion trap (ITD) analyser (Finnigan, San Jose, CA, USA). Positive atmospheric pressure chemical ionisation (+APCI) was applied in all performed experiments. The following experimental conditions were used during infusion of 50 µg mL^{-1} of each standard into the source (at the flow rate of 3 µL min^{-1}): capillary temperature 170 °C, vaporiser temperature 300 °C, flow rates of sheath gas and auxiliary gas 1.5 and 3 L min^{-1}, respectively, source voltage and current 6 kV and 7 mA, respectively. Specific conditions applied for the detection of

Table 2. MS detector setting in LC/MS–MS and GC/MS analyses

		Specification and conditions set for individual analytes				
Method of identification or quantification	Parameter	Bergapten, xanthotoxin (isobergapten, sphondin)	Angelicin, psoralen	Isopimpinellin (pimpinellin)	Trioxsalen	Bergamottin
LC/MS–MS	Capillary voltage (V)	22	31	19	8	46
	Activation amplitude (%)	37	25	35	38	35
	Activation Q	0.34	0.25	0.35	0.35	0.35
	Activation time (ms)	33	30	35	35	30
	Molecular ion (M^+), *m/z*	217.3	187.3	247.2	229.3	338.9
	Product ions (M^+), *m/z*	202.3	187.3[a]	232.2	142.3, 201.1, 173.2, 157.8	203.2
GC/MS (SIM)	EI ionisation (eV)	70	70	70	70	–
	Quantification ion, *m/z*	216	186	246	228	–
	Qualification ions, *m/z*	173, 201, 145	158, 130, 102	231, 188	199, 128, 185	–

[a] Due to low intensity of fragment ions, molecular ion was used for quantification.

J Sci Food Agric **87**:2152–2163 (2007)
DOI: 10.1002/jsfa

furanocoumarins are shown in Table 2. The mass analyser was programmed to perform a full scan in the *m/z* range of 50–500 in a positive mode for each analyte. Quantification was obtained by comparing the peak areas of the furanocoumarins with the corresponding calibration curve prepared for the standards.

GC/MS. An HP 6890 (Agilent Technologies) capillary gas chromatograph in conjunction with a 5973 mass selective detector (MSD) of the same producer was used. Furanocoumarins were separated on capillary column DB-5MS (60 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA, USA). The oven temperature was held at 75 °C for 2 min, then increased at the rate of 20 °C min^{-1} to 250 °C; held for 3 min and again increased at the rate of 30 °C min^{-1} to 280 °C and held for 5 min. One microlitres of sample was introduced onto the GC column in splitless mode. The injectior temperature was kept constant at 250 °C, and a splitless period of 2 min was used. MSD using an electron impact (EI) type of ionisation was operated in selected ion monitoring (SIM) mode. Quantification was achieved by comparing the peak areas of the target analytes with the abundances of these compounds in corresponding standards used for the calibration curve preparation. Table 2 lists ions used for the quantification and confirmation purposes when running GC/MS in the SIM mode.

RESULTS AND DISCUSSION

As summarised in the Introduction, a wide range of analytical procedures was utilised in studies concerned with the occurrence of furanocoumarins in various crops. Rather surprisingly, quality assurance/quality control (QA/QC) issues were not discussed in any of them. In the following paragraphs, two of the most commonly used procedures (HPLC and GC) and both their advantages and limitations are discussed on the basis of the data generated within the validation process.

Determination of furanocoumarins in vegetables (*Apiaceae*)

Regarding the extraction of furanocoumarins, several solvents largely differing in their polarity and other physico-chemical properties were used in the published studies. Since certified reference material (CRM) is currently not commercially available, the reliability of results cannot be easily assessed. To optimise the extraction step for the highest recovery of furanocoumarins, three solvents varying in polarities (water, ethyl acetate and dichlormethane) and one solvent mixture (acetonitril/water, 1:1), were tested in our experiments. As shown in Fig. 1, extraction with ethyl acetate was found to be the most efficient and, therefore, this solvent was used in all subsequent experiments.

Optimisation of identification and quantification

In the first step, HPLC separation of the available furanocoumarin standards was optimised. Using the reversed phase (RP) C18 silica separation column with the gradient of water and methanol as a mobile phase, angelicin, psoralen, bergapten, xanthotoxin, trioxsalen and isopimpinellin were easily separated. LODs ranged from 0.05 to 0.2 μg g^{-1} when employing a UV detector. However, when analysing extracts prepared from vegetables representing the *Apiaceae* family, interference from co-isolated matrix components with the peaks of major furanocoumarins was encountered. To improve the performance characteristics of the analytical method (the selectivity of analyte detection as well as the accuracy of the generated data) and to avoid the need to employ a purification step, a mass spectrometry detector (ion trap analyser) was used instead. The optimal ionisation of target analytes, hence the highest signal-to-noise (S/N) ratio was obtained by using positive APCI. Negative APCI and electrospray ionisation (ESI) were also tested within this study, but these ionisation techniques were much less sensitive. The analysis of the real-life parsnip sample is illustrated in Fig. 2 as an example. Considering the literature data[13] and comparing the spectral information and the relative retention

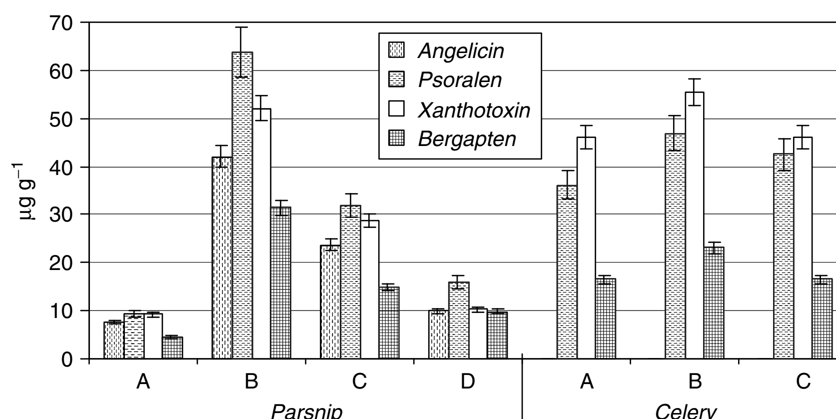

Figure 1. Efficiency of furanocoumarins extraction achieved using various solvents/solvent mixtures, quantification carried out by GC/MS (average values, $n = 3$); A = H_2O; B = ethyl acetate; C = CH_2Cl_2; D = acetonitrile/H_2O (1:1, v/v).

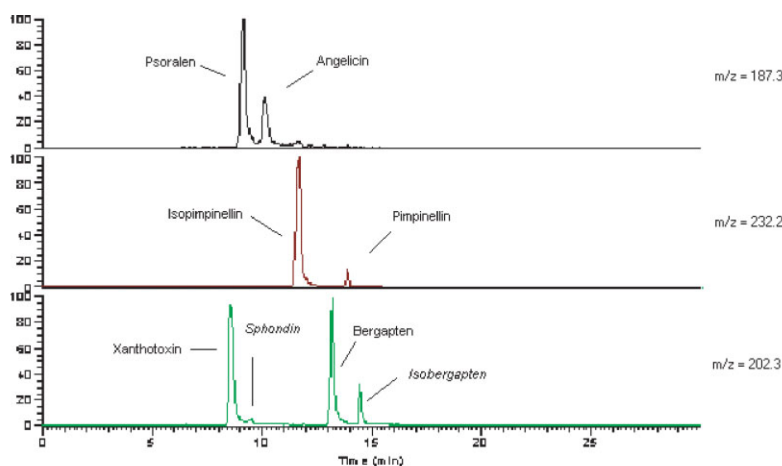

Figure 2. LC/MS (+APCI) analysis of furanocoumarins in extract from parsnip (aliquot contained in injected sample corresponded to 2 mg of the original matrix), content of individual analytes was 2.2 µg g^{-1} for psoralen, 1.8 µg g^{-1} for angelicin, 1.5 µg g^{-1} for isopimpinellin, 0.2 µg g^{-1} for pimpinellin, 7.1 µg g^{-1} for xanthotoxin, 0.7 µg g^{-1} for sphondin, 4.5 µg g^{-1} for bergapten and 2.0 µg g^{-1} for isobergapten; pimpinellin sphondin and isobergapten were identified only tentatively.

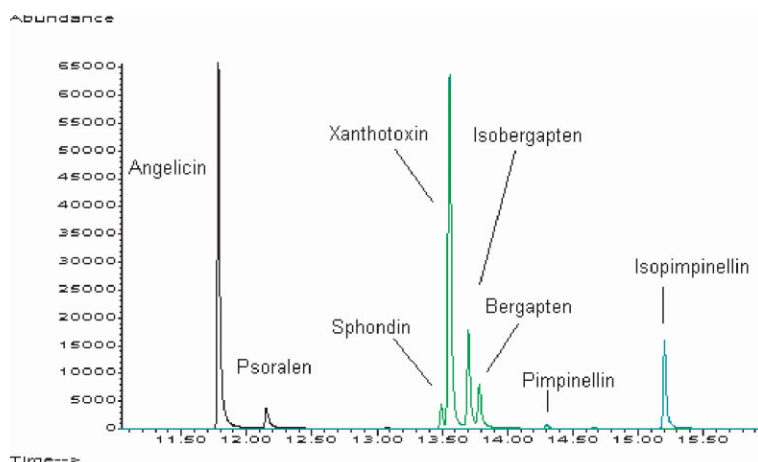

Figure 3. GC/MS analysis of furanocoumarins in extract from parsnip (aliquot contained in injected sample corresponded to 0.1 mg of the original matrix), content of individual analytes was 0.9 µg g^{-1} for psoralen, 6.2 µg g^{-1} for angelicin, 2.0 µg g^{-1} for isopimpinellin, 0.3 µg g^{-1} for pimpinellin, 7.1 µg g^{-1} for xanthotoxin, 0.7 µg g^{-1} for sphondin, 2.5 µg g^{-1} for bergapten and 4.5 µg g^{-1} for isobergapten; pimpinellin sphondin and isobergapten were identified only tentatively.

times, pimpinellin, sphondin and isobergapten were tentatively identified in our study. LODs for all analytes using MS detection were in the range 0.02–0.2 µg g^{-1}.

As a conceivable alternative, GC/MS procedure was optimised for the determination of angelicin, psoralen, bergapten, xanthotoxin, sphondin, isobergapten, trioxsalen, pimpinellin and isopimpinellin (see Fig. 3). In addition to identification by comparison of the measured data with the commercially available analytical standards, such as pimpinellin, sphondin and isobergapten, the target compounds were also identified from an NIST library search, while MSD was operated in the full scan mode. LODs for all analytes (MSD operated in SIM mode) were in the range 0.01–0.08 µg g^{-1} Limits of quantification (LOQs) were calculated as three times the levels of LODs.

Regarding the quantification of the analytes, the levels of furanocoumarins determined by either the GC/MS or LC/MS–MS method were comparable for the same parsnip and celeriac samples. The concentrations determined by the HPLC/UV method were rather higher for some of the target analytes (psoralen and angelicin). This was probably due to co-elution of some UV-absorbing matrix co-extracts. Since lower LODs were obtained, GC/MS method was preferred for identification and quantification of furanocoumarins in vegetables. The overview of the performance characteristic is summarised in Table 3.

Determination of furanocoumarins in citrus fruits and products containing bergamottin

To identify the optimal solvent for isolation of bergamottin (the major furanocoumarin in citrus fruits), the extraction efficiency of acetonitrile, methanol and/or ethyl acetate extraction was compared. As documented in Fig. 4, methanol was the most suitable solvent in this case; moreover, this solvent is compatible with the LC separation system. Contrary to vegetables representing the *Apiaceae* family

J Sci Food Agric **87**:2152–2163 (2007)
DOI: 10.1002/jsfa

Table 3. Performance characteristics of the LC/UV, LC/MS–MS and GC/MS methods employed for the determination of furanocoumarins

Characteristic of method			Furanocoumarin						
Method	Parameter	Matrix (spiking level)	Angelicin	Psoralen	Xanthotoxin	Bergapten	Isopimpinellin	Trioxsalen	Bergamottin
LC/UV	LOD ($\mu g\,g^{-1}$)	Vegetables (*Apiaceae*)	0.07	0.06	0.06	0.20	0.05	0.05	ND
LC/MS	LOD ($\mu g\,g^{-1}$)	Vegetables, citrus fruit	0.23	0.04	0.04	0.07	0.02	0.02	0.01
	Recovery (%) ($n = 3$)	Citrus pulp ($1\,\mu g\,g^{-1}$)	110	101	101	94	92	92	97
		Citrus peel ($1\,\mu g\,g^{-1}$)	111	111	101	104	102	100	a
	RSD (%) of method ($n = 8$)	Citrus pulp ($1\,\mu g\,g^{-1}$)	7	2	3	4	4	3	8
		Citrus peel ($1\,\mu g\,g^{-1}$)	7	4	6	4	2	2	a
GC/MS	LOD ($\mu g\,g^{-1}$)	Vegetables (*Apiaceae*)	0.02	0.08	0.03	0.04	0.02	0.01	ND
	Recovery (%) ($n = 3$)	Celeriac ($3\,\mu g\,g^{-1}$)	93	102	102	97	96	95	ND
		Parsnip ($10\,\mu g\,g^{-1}$)	93	93	97	92	94	95	ND
	RSD (%) of method ($n = 8$)	Celeriac ($0.1–16\,\mu g\,g^{-1}$)[b]	ND	5	5	5	8	ND	ND
		Parsnip ($1–10\,\mu g\,g^{-1}$)[b]	4	7	7	4	4	ND	ND

[a] Recovery not tested, the original content of bergamottin was $72\,\mu g\,g^{-1}$.
[b] Natural level.
ND, not detected.

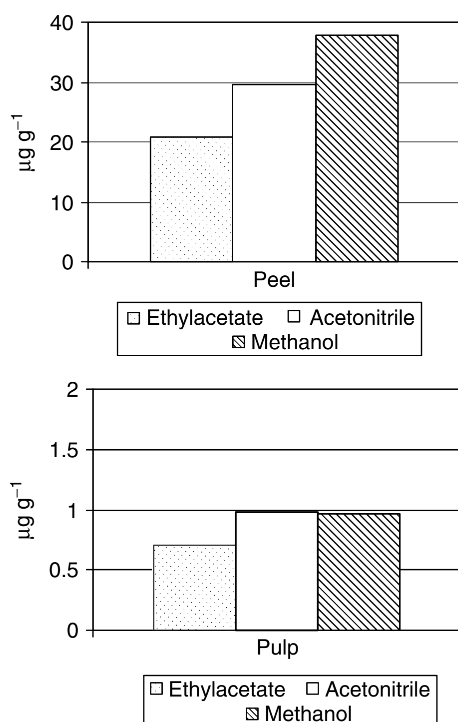

Figure 4. Comparison of extraction efficiencies for furanocoumarins in peel and pulp using various solvents (average values, $n = 3$).

where GC/MS method was the preferred alternative, LC/MS is the only applicable procedure for analysis of furanocoumarins in citrus fruit (see Fig. 5). As shown in our preliminary experiments,[23] degradation of bergamottin occurs under GC conditions even when a programmable temperature vaporisation injector (PTV, sample introduced into cold injector with temperature gradient $500\,°C\,min^{-1}$ starting at 40 °C) is used instead of hot splitless injector. The performance characteristics of the procedure optimised for citrus fruit and several other matrices are summarised in Table 3.

Levels of furanocoumarins in vegetables and fruits

Levels of furanocoumarins in vegetables

To generate the data needed for estimation of furanocoumarins dietary intake, average levels of these natural toxins were determined in samples collected at retail market. As shown in Table 4, the furanocoumarin levels determined were within the range reported for various crops and products in the literature.[2] It should be noted that the average levels of the relatively more toxic linear furanocoumarins (represented by xanthotoxin, bergapten and psoralen) in celeriac root and parsnip were comparable. In addition, angular furanocoumarins (angelicin, sphondin and isobergapten) were contained in parsnip in relatively high amounts as well. According to results obtained in animal experiments,[2] these compounds demonstrate relatively lower toxicity. One should be aware that considering the values of 'total' furanocoumarins for the dietary risk assessment might be rather misleading, the content of individual compounds should always be specified.

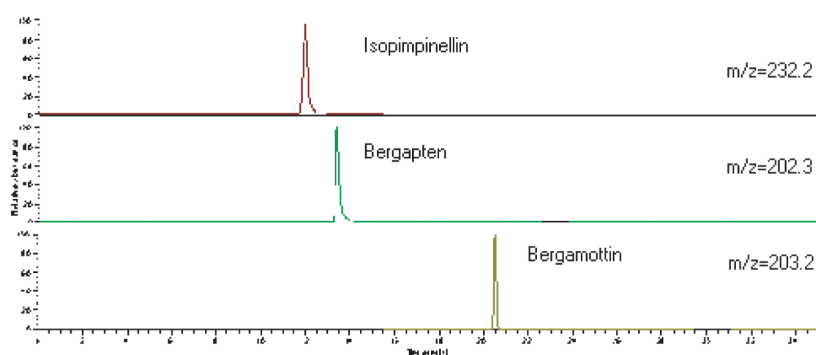

Figure 5. LC/MS analysis of furanocoumarins in extract from lime pulp (aliquot contained in injected sample corresponded to 2 mg of the original matrix); content of individual analytes was 0.8 $\mu g\ g^{-1}$ for isopimpinellin, 0.3 $\mu g\ g^{-1}$ for bergapten and 5.8 $\mu g\ g^{-1}$ for bergamottin.

Table 4. Average levels of furanocoumarins in food products obtained at a Czech market ($\mu g\ g^{-1}$), compared with the results from another study

	Vegetable					
	Parsnip (*Pastinaca sativa*)		Celeriac root (*Apium graveolens*)		Parsley (*Petroselinum sativum*)	
Furanocoumarin	Our study ($n = 50$)	Published data[1]	Our study ($n = 50$)	Published data[1]	Our study ($n = 50$)	Published data[1]
Linear						
Psoralen	<LOD–6.6	0.1–10.5	<LOD–5.8	0.1–10.5	<LOD–0.1	0.1–0.5
Xanthotoxin	1.1–28.0	0.8–48.0	1.2–9.7	0.4–22.0	0.1–0.3	0.3–1.4
Bergapten	0.9–9.0	0.9–7.0	1.5–5.9	0.7–31.5	0.2–1.7	1.0–9.0
Isopimpinellin	0.7–8.3	1.4–12.6	1.1–10.7	1.4–12.6	0.1–0.3	2.3
Trioxsalen	<LOD	NR	<LOD	NR	<LOD	NR
Angular						
Angelicin	0.4–27.8	1.8–20.8	<LOD	NR	<LOD	NR
Sphondin[a]	0.2–4.9	NR	<LOD	NR	<LOD	NR
Isobergapten[a]	1.0–16.3	NR	<LOD	NR	<LOD	NR
Pimpinellin[a]	<LOD–0.7	NR	<LOD	NR	<LOD	NR
Furanocoumarin content						
Total	**5–89**	**1–140**	**4–38**	**1.1–50**	**0.3–2.4**	**1.3**
Average	**26.2**	NR	**17.3**	NR	**1.4**	NR
Median	17.6	NR	16.4	NR	1.4	NR
RSD (%)	77	NR	56	NR	111	NR
Percentile 0.1	8.5	NR	7.4	NR	0.5	NR
Percentile 0.9	56.8	NR	30.8	NR	2.2	NR

n = number of examined samples.
NR = not reported in the published study.
[a] tentative identification
[1] Søborg *et al*., 1996.
Values in bold type are sum of all analysed furanocoumarins.

To identify potential differences in furanocoumarin levels, various celeriac root cultivars were analysed in the next part of our study. As shown in Fig. 6, their profiles were very similar, while bergapten was the dominating furanocoumarin occurring in celeriac root. Rather surprisingly, psoralen was not detected in both cultivars Maxim and Neon, although this compound was unambiguously found in the other two cultivars.

Levels of furanocoumarins in vegetable products from a Czech market

In addition to fresh vegetables, several other products available at the market containing *Apiaceae* vegetables were examined for the presence of furanocoumarins. Table 5 shows the content of furanocoumarins (average value obtained by analyses of the representative sample prepared from five individual packages) in seasoning, soup, salad and frozen vegetable mixture. Relatively low levels of furanocoumarins were detected in frozen vegetable mixtures (containing celeriac root, carrot, parsnip or parsley), these relatively high concentrations (50 $\mu g\ g^{-1}$ and greater) were found in fresh mixtures made of cut vegetables.

Levels of furanocoumarins in fruits containing bergamottin

Citrus fruits represent another dietary source of furanocoumarins. As shown in Table 6, the highest

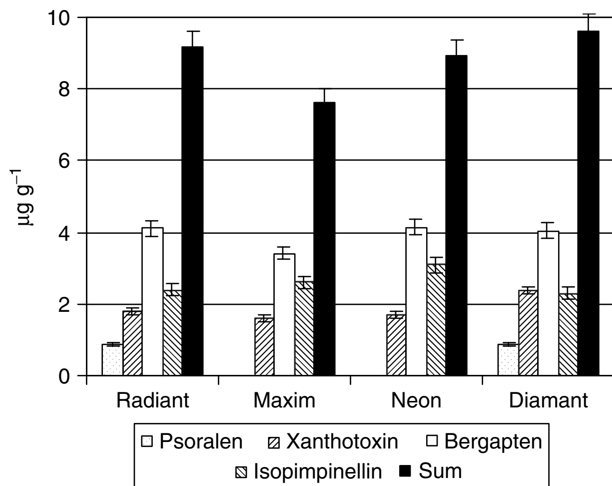

Figure 6. Comparison of furanocoumarin levels in harvested celeriac roots (different cultivars compared).

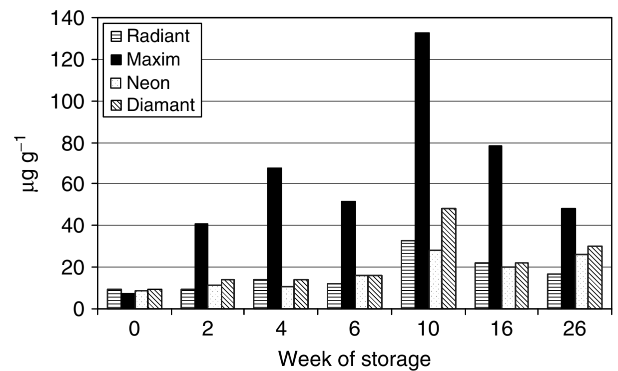

Figure 7. Changes of furanocoumarin levels during storage of celeriac root in household cellar (sum of furanocoumarins; $\mu g\,g^{-1}$; results expressed on a fresh weight basis, levels in dry matter did not differ significantly).

content of these natural toxins is contained in lemons and limes. As regards distribution of furanocoumarins within the fruit, peels contain largest levels of furanocoumarin (up to 50% of the total content). Although, typically, peels of citrus fruits are removed before consumption or industrial production of fruit juices, this waste material is commonly used for the isolation of essential oils. This product is widely applied in flavouring of various foodstuffs and, consequently, co-isolated furanocoumarins are transferred into the respective product. For example, high contents of bergamottin were found in some flavoured teas (Table 6). The additional experiments concerned with leaching of furanocoumarins into the infusion during the tee preparation showed that approximately 65% of their original content was transferred from 2 g of tea leaves into 100 mL of boiling water within 5 min. The total amount of furanocoumarins in the suspension (leached tea leaves + infusion) did not change in this experiment, which documents their thermal stability.

The dynamics of furanocoumarin levels in stored and processed celery and celeriac

In general, various physiological processes take place in food crops after harvesting,[11] both biodegradation and/or biosynthesis of secondary metabolites may occur. Since the *Apiaceae* crops are often consumed after several weeks or months after harvesting, the influence of storage conditions on furanocoumarin levels in selected samples was investigated.

Celeriac root

In this part of study, the levels of furanocoumarins were monitored during 26-week storage period in four varieties of celeriac root. As shown in Fig. 7, all celeriac root cultivars stored in a home cellar (experiment 1 in Table 1) showed a gradual increase of toxins, occurring within 10 weeks of storage. The peak concentration for all varieties was achieved in week 10. At that time, the levels of furanocoumarins in the Maxim cultivar were 16 times higher (the dominating representative being xanthotoxin) as compared to those determined right after harvesting. In the following period, a successive decrease of furanocoumarin levels took place and after 26 weeks of storage, their concentrations approached the original levels. It should be noted that, regardless the cultivar, while only small variations in furanocoumarin content were found for celeriac directly after the harvest (RSD = 7%, based on analysis of 10 individual roots), the differences of the concentrations were much higher at the end of the storage period (RSD = 53%).

Only slightly higher levels of furanocoumarins were found in celeriac root cultivars after storage in refrigerator (4 °C) for the period of 12 weeks (Fig. 8; and for experimental conditions see experiment 4 in Table 1).

As documented in several other studies,[2,11,17] the increase/decrease of toxic secondary metabolites in food crops occurs as a consequence of the damage of plant tissues. As can be seen from Fig. 9, rapid increase of furanocoumarins was observed in cut crops when compared to the intact celeriac roots (for experimental conditions see experiment 3 in Table 1). In contrast to celeriac, the changes in sliced parsnip[11] were less pronounced under the same conditions.

Celeriac haulm and celery

Although the differences in furanocoumarin levels after 4 week of celeriac roots storage at 4 °C were low (see Fig. 8), enormous increase of their concentration was found in celery and celeriac haulm after 4 weeks of storage in refrigerator (Fig. 10; for experimental conditions see experiments 4 and 5 in Table 1). An great increase in psoralen, as high as two orders of magnitude (originally $4\,\mu g\,g^{-1}$ d.w. as compared to $470\,\mu g\,g^{-1}$ d.w. after being stored), occurred in cultivar Maxim after 2 weeks of storing at these conditions. The increasing trend in concentrations of other furanocoumarins was also observed, although this trend was less intense (the levels of isopimpinellin and bergapten were only five times higher after 1 week of storage). Similar trends were reported by

Table 5. Furanocoumarins in vegetable products obtained at a Czech market, average content ($\mu g\, g^{-1}$ of original material) obtained by analysis of five different batches

Type of product	Commercial name	Description (composition)	Psoralen	Xanthotoxin	Bergapten	Isopimpinellin	Trioxsalen	Angelicin	Sphondin[a]	Isobergapten[a]	Pimpinellin[a]	Sum
Mixture of cut vegetables	Fresh salad	Fresh (carrot 50%, parsley 25%)	0.12	1.68	0.59	0.41	0.01	1.18	0.47	1.28	<LOD	5.75
	Soup mixture	Fresh, (carrot 30%, celeriac 20%, parsnip 30%)	17.39	15.90	17.07	4.27	0.01	<LOD	<LOD	<LOD	<LOD	54.65
	Vegetable soup	Frozen, (carrot 10%, celeriac 20%, parsnip 30%)	0.03	0.17	0.10	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	0.31
	Vegetable mixture	Frozen, (carrot 10%, celeriac 10%, parsley 20%)	0.08	0.37	0.30	0.13	<LOD	0.10	0.02	0.13	<LOD	1.14
Dry vegetable	Vegetable mixture	Seasoning	1.25	26.58	5.20	4.46	<LOD	27.60	<LOD	<LOD	<LOD	65.08
	Parsley haulm	Seasoning	0.77	1.04	34.47	2.86	<LOD	<LOD	<LOD	<LOD	<LOD	39.14
	Parsley haulm	Seasoning	<LOD	<LOD	23.27	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	23.27
Seasoning	Podravka	Seasoning for soup	0.21	0.52	0.91	0.40	<LOD	0.29	0.04	0.14	<LOD	2.51
	Ascommerce	Seasoning for soup	0.67	0.58	0.68	0.27	<LOD	0.12	0.21	0.10	<LOD	2.63
	Tant	Seasoning for soup	0.06	0.10	0.55	0.20	<LOD	0.05	<LOD	0.02	<LOD	0.97
Juice	Vegetable juice	(Carrot 25%, celeriac 25%, parsnip 25%, parsley 25%)	0.04	0.03	0.03	0.03	<LOD	<LOD	<LOD	<LOD	<LOD	0.13
Processed celery	Sterilised celeriac	Solid portion (33% w/w)	0.05	0.28	0.43	0.23	<LOD	<LOD	<LOD	<LOD	<LOD	0.99
		Liquid part (67%, w/w)	<LOD	0.08	0.08	0.06	<LOD	<LOD	<LOD	<LOD	<LOD	0.22

[a] tentative identification.

J Sci Food Agric **87**:2152–2163 (2007)
DOI: 10.1002/jsfa

Table 6. Furanocoumarins in fruits and fruit products (average, $n = 5$) obtained at a Czech market, average content ($\mu g\ g^{-1}$)

Product	Description[a]	Psoralen	Xanthotoxin	Bergapten	Isopimpinellin	Bergamottin	Sum
Fresh fruit and juices							
Lemon	Pulp (80%)	<LOD	<LOD	<LOD	<LOD	0.25	0.3
	Peel (20%)	<LOD	<LOD	2.57	0.14	72.27	75.0
Grapefruit	Pulp (76%)	<LOD	<LOD	<LOD	<LOD	2.96	3.0
	Peel (24%)	<LOD	<LOD	1.92	<LOD	10.18	12.1
Lime	Pulp (83%)	<LOD	<LOD	0.35	0.98	6.02	7.4
	Peel (17%)	0.12	0.38	12.91	4.49	33.40	51.3
Mandarin	Pulp (82%)	<LOD	<LOD	<LOD	<LOD	0.05	0.1
	Peel (18%)	<LOD	<LOD	<LOD	<LOD	0.52	0.5
Orange	Pulp (75%)	<LOD	<LOD	<LOD	<LOD	0.05	0.1
	Peel (25%)	<LOD	<LOD	<LOD	<LOD	0.52	0.5
Grapefruit juice	100% natural	<LOD	<LOD	0.001	0.002	0.098	0.1
Orange juice	100% natural	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Rio Bio Activ juice	100% natural	<LOD	<LOD	<LOD	0.330	0.290	0.6
Teas							
Pickwick lemon tea	Lemon peel	<LOD	<LOD	<LOD	<LOD	5.4	5.4
Pickwick Earl Grey tea	Flavour	<LOD	<LOD	<LOD	<LOD	0.1	0.1
Fruit tea	Orange peel	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fruit tea	Lemon peel	<LOD	<LOD	<LOD	0.1	3.4	3.5
Fruit tea	Grapefruit peel	<LOD	<LOD	0.1	<LOD	3.1	3.2
Tea Earl Grey	(Pink teahouse)	0.1	<LOD	0.6	0.1	16.9	17.6
Tea Earl Grey	(Teekanne)	0.1	0.2	0.4	<LOD	44.7	45.4
Green Tea	Grapefruit peel	<LOD	<LOD	<LOD	<LOD	0.2	0.2
Green tea	Lemon peel	0.1	0.1	0.1	0.3	2.6	3.3

[a] Average proportion from the total weight (%).

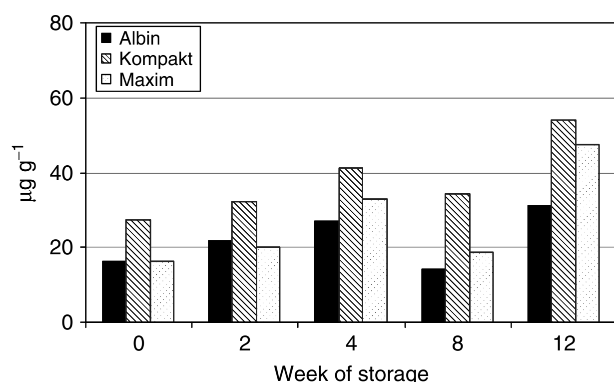

Figure 8. Changes of furanocoumarin levels in celeriac roots during storage at 4 °C (sum of furanocoumarins; $\mu g\ g^{-1}$).

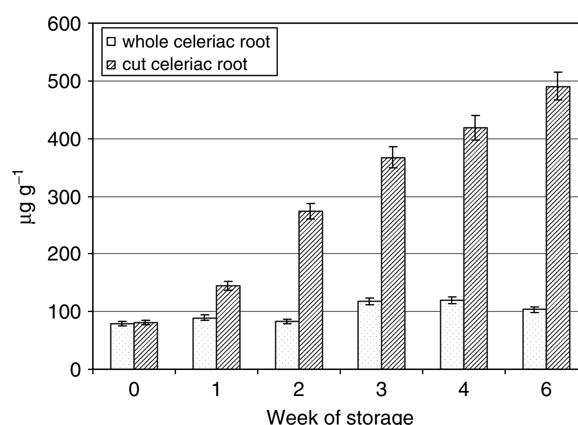

Figure 9. Changes of furanocoumarin levels in whole and cut celeriac root (cultivar Radiant) during storage at 4–8 °C in a cellar (total content, $\mu g\ g^{-1}$).

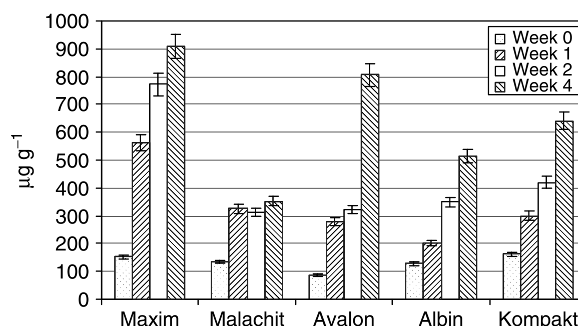

Figure 10. Changes of furanocoumarins in celery and celeriac haulms during storage at 4 °C (total content, $\mu g\ g^{-1}$ dry weight).

Chaudhary:[17] levels of furanocoumarins increased four times after 44 days of storage at 4 °C.

In addition to the storage experiments discussed above, homogenised samples of whole haulm were also analysed. Furanocoumarin levels in homogenates varied only slightly during storage at 4 °C as compared to the content of furanocoumarins determined in the haulm.

Dry celery and celeriac haulm are often used in seasoning mixtures. In our study, a slight decrease (e.g. 118 $\mu g\ g^{-1}$ d.w., originally 152 $\mu g\ g^{-1}$ d.w. in Maxim cultivar, or 63 $\mu g\ g^{-1}$ d.w., originally 144 $\mu g\ g^{-1}$ d.w. in Malachit cultivar) of furanocoumarin concentrations was found (for experimental conditions see experiments 4 and 5 in Table 1). Similar experiments were not described in any of the published studies.

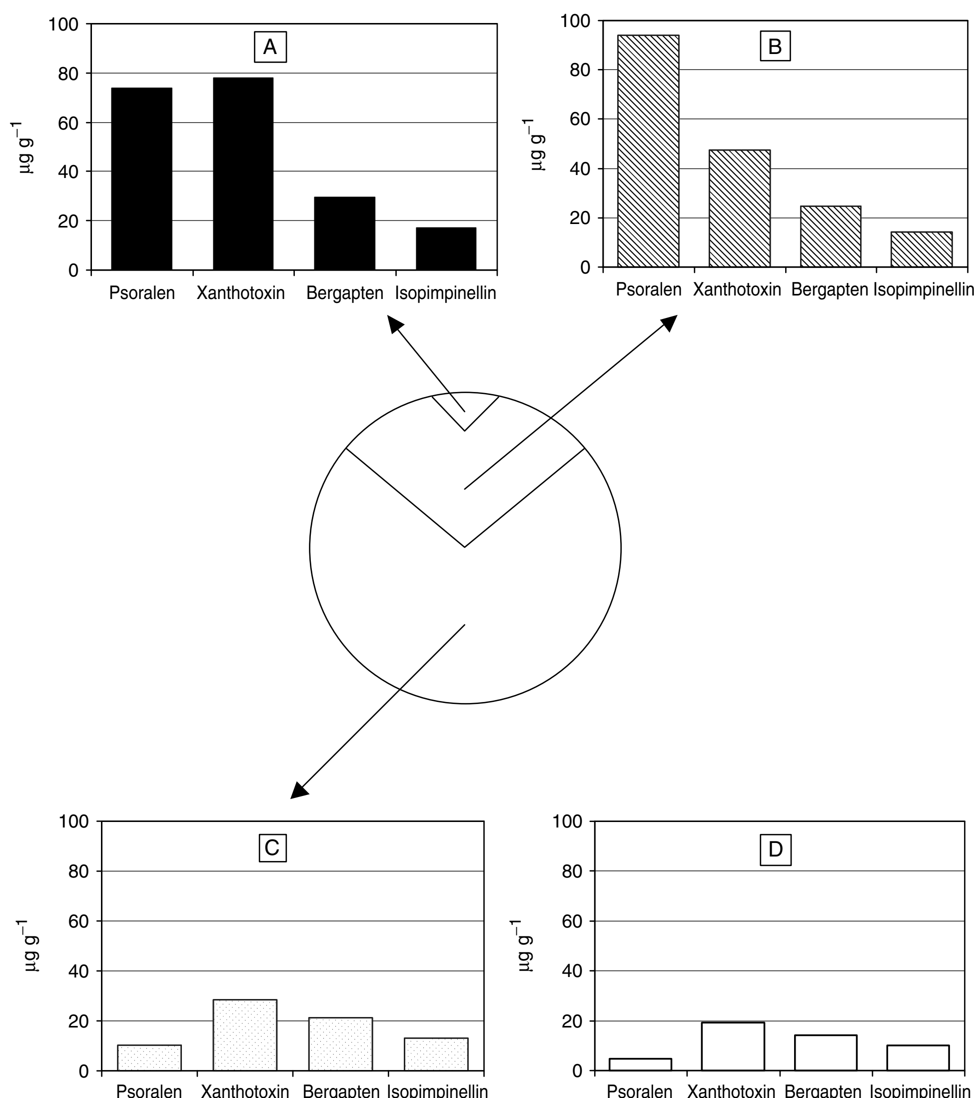

Figure 11. Distribution of furanocoumarins in celeriac root infected by fungi. A = infected tissue, B = areas adjacent to rotten tissue, C = healthy tissue (visually inspected), D = healthy (uninfected) celeriac root.

Distribution of furanocoumarins in damaged celeriac root of Maxim cultivar

The influence of damage caused by fungal infection on the content of furanocoumarins in celeriac roots was investigated (for experimental conditions see experiment 2 in Table 1). Levels of furanocoumarins in parts infected by fungi and areas adjacent to the affected parts of celeriac root rapidly increased to levels as high as 200 and 180 $\mu g\,g^{-1}$, respectively. Higher levels of furanocoumarins (76 $\mu g\,g^{-1}$) were also found in healthy areas (the remaining three quarters of the damaged root, visually inspected) as compared to healthy celeriac roots with average levels of only 48 $\mu g\,g^{-1}$ (Fig. 11). The results are comparable with those reported in the literature,[11] where levels of furanocoumarins were 10 times higher in affected celery as compared to healthy celery.

CONCLUSIONS

As demonstrated in this study, both GC/MS and LC/MS methods enable accurate identification and quantification of major furanocoumarins occurring in *Apiaceae* and *Rutaceae* food crops. With the exception of citrus fruit, the GC/MS method is the method of choice to examine the furanocoumarin content in plant matrices, because the crude ethyl acetate extract can be analysed directly, while an exchange of solvent is needed prior to LC/MS analysis. In addition, identification is enabled when using GC in comparison to the mass spectra obtained with the NIST library. This is especially important for the minor furanocoumarins, for which commercial standards are not available yet.

The average levels of furanocoumarins determined in fresh celeriac roots (17 $mg\,kg^{-1}$) and parsnips (26 $mg\,kg^{-1}$) collected at a Czech market were comparable to those reported in other similar studies; nevertheless, a large variability in toxins levels was shown (1–50 and 1–140 $mg\,kg^{-1}$, respectively).

Storage conditions were found to play an important role in the nature of the change in furanocoumarins (for both total content and the pattern of respective compounds) during the storage time. In this respect,

J Sci Food Agric **87**:2152–2163 (2007)
DOI: 10.1002/jsfa

temperature is the key factor. In general, storing the root vegetable below 4 °C prevents and increase in furanocoumarin levels, which may occur when the crop is kept at higher temperatures. In contrast to the root parts, a rapid increase of furanocoumarins was observed in haulms (even when refrigerated).

In order to reduce/minimise the dietary intake of furanocoumarins and avoid any health risk associated with the intake of these natural toxins, the consumption of injured vegetables should be totally avoided. Care should be also taken to store vegetables properly, i.e. at rather low temperatures. Significant reduction of the furanocoumarin content can be achieved by peeling the vegetable carefully.

ACKNOWLEDGEMENT

This study was carried out within the project MSM No. 6046137305 supported by the Ministry of Education, Youth and Sports of the Czech Republic. Implementation of analytical procedure was funded by project COST 924. The authors wish to express their thanks to Dr Christer Andersson from National Food Administration, Uppsala, Sweden for scientific advice on the project.

REFERENCES

1 Sardari S, Mori Y, Horita K, Micetich RG, Nishibe S and Daneshtalab M, Synthesis and antifungal activity of coumarins and angular furanocoumarins. *Bioorg Med Chem* **7**:1933–1940 (1999).
2 Søborg I, Andersson C and Gry J, *TemaNord: Furanocoumarins in Plant Food*. Copenhagen, Denmark (1996).
3 Llano J, Raber J and Eriksson LA, Theoretical study of phototoxic reactions of psoralens. *J Photochem Photobiol A* **154**:235–243 (2003).
4 Järvenpää EP, Jestoi MN and Huopalahti R, Quantitative determination of phototoxic furanocoumarins in celeriac (*Apium graveolens* L. Var. Rapeceum) using supercritical fluid extraction and high performance liquid chromatography. *Phytochem Anal* **8**:250–256 (1997).
5 Diawara MM, Trumble JT, Quiros CF and Hensen RJ, Implications of distributions of linear furanocoumarins within celery. *J Agric Food Chem* **43**:723–727 (1995).
6 Lombeart GA, Sieements KH, Pellars P, Mankotia M and Ng W, Furanocoumarins in celery and parsnip. *JAOAC International* **84**:1135–1143 (2001).
7 Bier RC, Ivie BW and Oertli EH, Linear furanocoumarins and graveolone from the common herb parsley. *Phytochemistry* **36**:869–872 (1994).
8 Fukuda A, Guo L, Ohaski N, Yoshikawa M and Yamazoe Y, Amount and variation in grapefruit juice of the main components causing grapefruit–drug interaction. *J Chromatog B* **741**:195–203 (2000).
9 Murray RDH, Mendez J and Brown SA, *The Natural Coumarins*, Wiley, Chichester, UK (1982).
10 Health-Pagliuso S, Matlin SA, Fang N, Thompson RH and Rappaport L, Simulation of furanocoumarin accumulation in celery and celeriac tissue by *Fusarium oxysporum* F. SP. Apii. *Phytochemistry* **31**:2683–2688 (1992).
11 Ostertag E, Becker T, Ammon J, Buer-Aymanns H and Schrenk D, Effect of storage conditions on furanocoumarin levels in intact, chopped, or homogenized parsnips. *J Agric Food Chem* **50**:2565–2570 (2002).
12 Ivie GW, Holt DL and Ivey MC, Natural toxicants in human foods: Psoralens in raw and cooked parsnip root. *Science* **213**:909–9101 (1981).
13 Cardoso CAL, Vilegas W and Honda NK, Rapid determination of furanocoumarins in creams and pomades using SPE and GC. *J Pharm Biomed Anal* **22**:203–214 (2000).
14 Zgorka G and Glowniak K, Simultaneous determination of phenolic acids and linear furanocoumarins in fruits of *Libanotis dolichostyla* by solid-phase extraction and high performance liquid chromatography. *Phytochem Anal* **10**:268–271 (1999).
15 Berenbaum MR, Zangerl AR and Nitao JK, Furanocoumarins in seeds of wild and cultivated parsnip. *Phytochemistry* **23**:1809–1810 (1984).
16 Spencer GF, Tjarks LW and Powell RG, Analysis of linear and angular furanocoumarins by dual-column high performance liquid chromatography. *J Agric Food Chem* **35**:803–805 (1987).
17 Chaudhary SK, Ceska O, Warrington PJ and Ashwood-Smith MJ, Increased furocoumarin content of celery during storage. *J Agric Food Chem* **33**:1153–1157 (1985).
18 Ceska O, Chaudhary SK, Warrington PJ and Ashwood-Smith MJ, Furanocoumarins in the cultivated carrot, daucus carota. *Phytochemistry* **25**:81–83 (1986).
19 Zschocke S, Liu JH, Stuppner H and Bauer R, Comparative study of roots of *Angelica* sinensis and related umbelliferous drugs by thin layer chromatography, high-performance liquid chromatography, and liquid chromatography-mass spectrometry. *Phytochem Anal* **9**:283–290 (1998).
20 Dugo P, Mondello L, Dugo L, Stancanelli R and Dugo G, LC–MS for the identification of oxygen heterocyclic compounds in citrus essential oils. *J Pharm Biomed Anal* **24**:147–154 (2000).
21 Franke K, Porzel A, Masaoud M and Adam G, Furanocoumarins from *Dorstenie gigas* J. Schmidt. *Phytochemistry* **56**:611–621 (2001).
22 Schulzová V, Peroutka R and Hajšlová J, Levels of furanocoumarins in vegetables from organic and conventional farming, *Pol J Food Nutr Sci* **11/52**:25–27 (2002).
23 Su CFH and Horvath R, Isolation and characterization of four major components from insecticidally active lemon peel extract. *J Agric Food Chem* **35**:509–511 (1987).