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Evaluation of analytical markers characterising different drying methods of parsley leaves (*Petroselinum crispum* L.)

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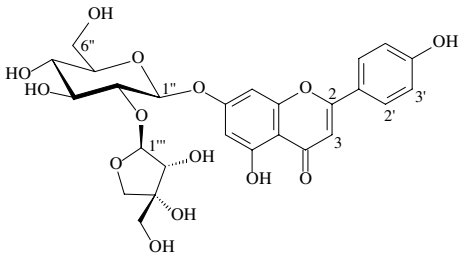
Drying process of parsley leaves from *Petroselinum crispum* L. can influence the sensory qualities and aromatic taste of this herbal product. Beside oven-dried material, freeze-dried parsley is getting increasingly into the market. In the course of a search for analytical tools to differentiate oven-dried and lyophilised parsley, a HPLC determination of the 6″-*O*-malonylapiin to apiin ratio was shown to be a suitable marker system. While the ratio is high for fresh and lyophilised leave material, oven-drying leads to demalonylation and, subsequently, to a low malonylapiin – apiin ratio. Additionally, L*a*b colour measurement can be used for quality control to differentiate between different dried parsley raw materials.

1. Introduction

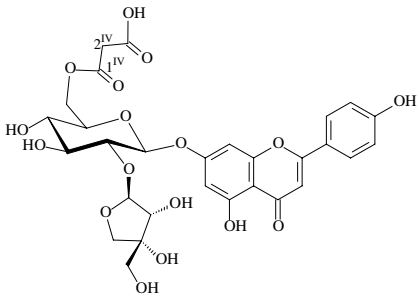
Parsley leaves from *Petroselinum crispum* ssp. *crispum* L. (syn. *P.c.* ssp. *foliosum* ALEF.) are a typical spice produced mainly in European countries with moderate climate (Germany, France, Balkan countries, Russia) but also in certain districts of China and other East Asian countries. For review on botany, phytochemistry and applications of parsley see Hager's Handbuch (Hänsel et al. 1994). Besides the traditional function in food technology the development of parsley extracts for anti-diabetic use is getting more into the focus (Yanardag et al. 2003; Bolkent et al. 2004; Ozsoy-Sacan et al. 2006). Parsley extracts are also used because of their phytoestrogen activity (Yoshikawa et al. 2000) and clinical trial samples have to be investigated analytically for release for preclinical and clinical studies (Meyer et al. 2006; Nielsen et al. 1999). For this the exact analytical characterisation of the leaf material for quality control is mandatory. During such investigations it was observed that parsley leaf material dried under different conditions exerts different flavour smell and differs in main sensoric parameters. These sensoric differences were also obvious in extracts prepared.

The typical flavour of the leaves is due to the essential oil with myristicin, apiol and elemicin as the main constituents and 1,3,8-menthatriene as the component causing the typical parsley flavour (Diaz-Maroto et al. 2002; Simon and Quinn 1988). On the other hand, it has to be considered that different chemocultivars with a variable quantitative composition are existing (myristicin cultivar, apiol cultivar and allyltetramethoxybenzene cultivar). Other constituents of the leaves are polyacetylenes (Zidorn et al. 2005), coumarins (up to 120 ppm) besides a variety of different flavone glycosides (2 to 6%) mainly apiin (**1**), 6″-*O*-malonylapiin (**2**) (Eckey-Kaltenbach et al. 1983), luteolin-7-apiosylglucoside and 6″-*O*-acetylapiin (Yoshikawa et al. 2000; Luthria et al. 2006).

On the other hand, the typical parsley flavour is also dependent on the way of technological preparation of the leaf material. Especially the drying process of parsley leaves can result in diminished flavour and aroma or leading to an aroma defect known as "hay flavour" (Masanetz and Grosch 1998). Alternatively, to produce high-quality (and therefore high-cost) products and to reduce the risk



1 Apiin



2 6-″-*O*-Malonylapiin

of flavour-changes during drying, lyophilised products with aromatic sensations comparable to that from native parsley are marketed more and more. Because of the higher pricing of lyophilised material it can be assumed that intermixtures with oven-dried parsley can occur or that air-dried leaves are marketed under the label "lyophilised material". For that reason, an exact analytical method for unknown samples allowing an unambiguous differentiation of lyophilised and oven-dried (40–70 °C) parsley leaves is desirable. The present study was performed to investigate different analytical methods and to establish a suitable protocol to characterise the used drying method.

2. Investigations and results

Fresh parsley leaves from different sources were divided into different lots and dried under controlled conditions, either by lyophilisation under standard laboratory conditions or by oven-drying at 45 or 70 °C. It was not possible to dry at ambient temperature because plant material wilted or got mouldy. The resulting leave material obtained differed obviously concerning colour and intensity of flavour. While freeze-dried material had a more intense green colour and a more "fresh" aromatic sensation, the oven-died material gave the optical impression of a slightly greyish colour and a non-intense sensory impact. On the other hand it was not possible to differentiate mixtures of oven-dried and lyophilised material only by optical and sensoric inspection. In order to evaluate other criteria for analytical differentiation of oven-dried and lyophilised parsley, microscopic investigation of the leaves was performed: the typical up- and downside epidermis was obvious, containing stomata with 3 to 4 accessory cells. Typical cuticular bands were obvious. No significant difference was found between products originating from different drying processes.

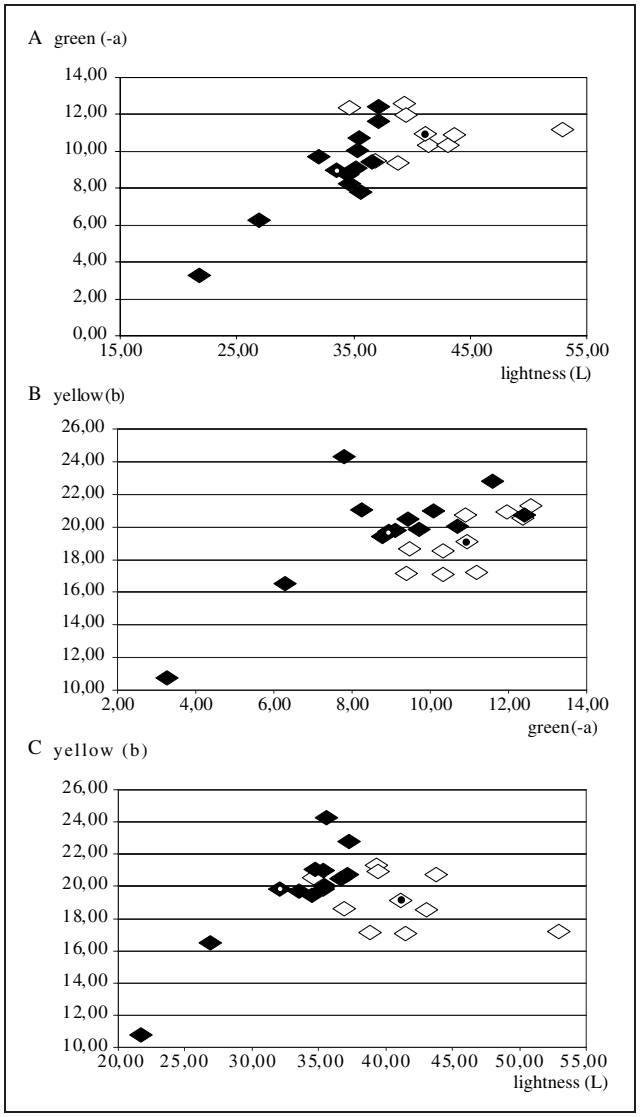


Fig. 1: L^*a^*b correlations of oven-dried and lyophilised parsley samples. Oven-dried samples: black hashes, lyophilised samples: white hashes; dots in hashes: mean values. A: L (lightness) vs. (-a) (green); B: (-a) (green) vs. b (yellow); C: L (lightness) vs. b (yellow). Samples originated from different sources and were either lyophilised or oven-dried under same conditions.

In order to focus on the sample colouration, L^*a^*b colour space measurements were performed. As shown in Fig. 1A, a clear tendency between the two groups of samples from different drying processes became obvious: a higher degree of lightness was mainly found within clusters of the lyophilised samples (Figs. 1A and 1C) while the oven-dried leaves had the tendency to be grouped within yellow clusters (Fig. 1B); freeze-dried material clustered dominantly in the green-region (1A). On the other hand it had to be considered that the measured variability in L^*a^*b was not only due to the drying process applied on the leaves, but was additionally influenced by the differences of the respective raw material in use.

Because temperature-exposed plant material should have a different level of active enzymes, the semi-quantitative ApiZYM® test was used in order to determine a wide variety of enzyme activities (esterases, glycosidases, peptidases, proteases etc.) in aqueous extracts of different dried parsley leaves. From 20 enzymes tested, 19 did not differ significantly between lyophilised leaves and material oven-dried at 45 and 70 °C. Only a trypsin-like protease was active in freeze-dried material, while the respective air-dried samples had significant lower activity levels of this enzyme.

Also determination of the volatile oil after steam distillation indicated that the absolute oil content (about 0.1 to 0.2%) is not strictly dependent on the drying method. Also GC-MS analysis of the essential oil (Simon and Quinn 1988) and quantification of the main oil compounds confirmed this: no clear correlation could be made between leaf material and drying process (Table). Two samples from different origins were lyophilised or oven-dried at 45 resp. 70 °C. The results indicated that even drying at high temperatures does not necessarily lead to diminished volatile oil contents.

In order to assess the overall chemical similarity between the various samples, ^{1}H NMR spectra of crude methanol extracts from parsley leaves were recorded and submitted to principal component analysis (PCA). The major part (92%) of the variance between the 11 parsley samples investigated was explained by the first two principal components (Fig. 2). It was found that this method discriminated reasonably well between parsley leaf samples from different geographic origins but did not allow a distinction between the different drying methods applied on these samples.

HPLC analysis of a methanolic extract revealed two peaks (**1** and **2**), which were clearly different between parsley leaves produced by different drying techniques: while peak **2** was dominant in fresh and lyophilised material, its intensity decreased significantly during oven-drying process, accompanied by an increase of peak **1** (Fig. 3).

Table: Quantification of the main essential oil components (mg/100 g) in two parsley samples dried under different conditions, either by freeze-drying (FD) or by oven-drying (OD) at 45 °C resp. 70 °C

Compd.	Rt (min)	Sample 1			Sample 2		
		FD	OD 45 °C	OD 70 °C	FD	OD 45 °C	OD 70 °C
α-Pinene	4.27	n.d.	0.31	0.08	0.47	1.70	2.53
β-Myrcene	5.35	1.75	14.34	2.07	0.50	0.50	0.36
α-Phellandrene	7.72	0.25	2.07	0.33	0.39	0.36	0.41
Terpinolene + p-cymenene	8.05	0.27	2.60	0.63	0.96	0.92	0.75
1,3,8-Menthatriene	8.81	0.06	1.79	0.25	4.67	2.59	5.16
Myristicin	22.09	61.10	88.54	57.99	33.61	27.32	29.60
Apiol	24.86	30.47	41.44	28.08	0.17	0.16	0.37
Sum		93.9	151.09	89.43	40.77	33.55	39.18

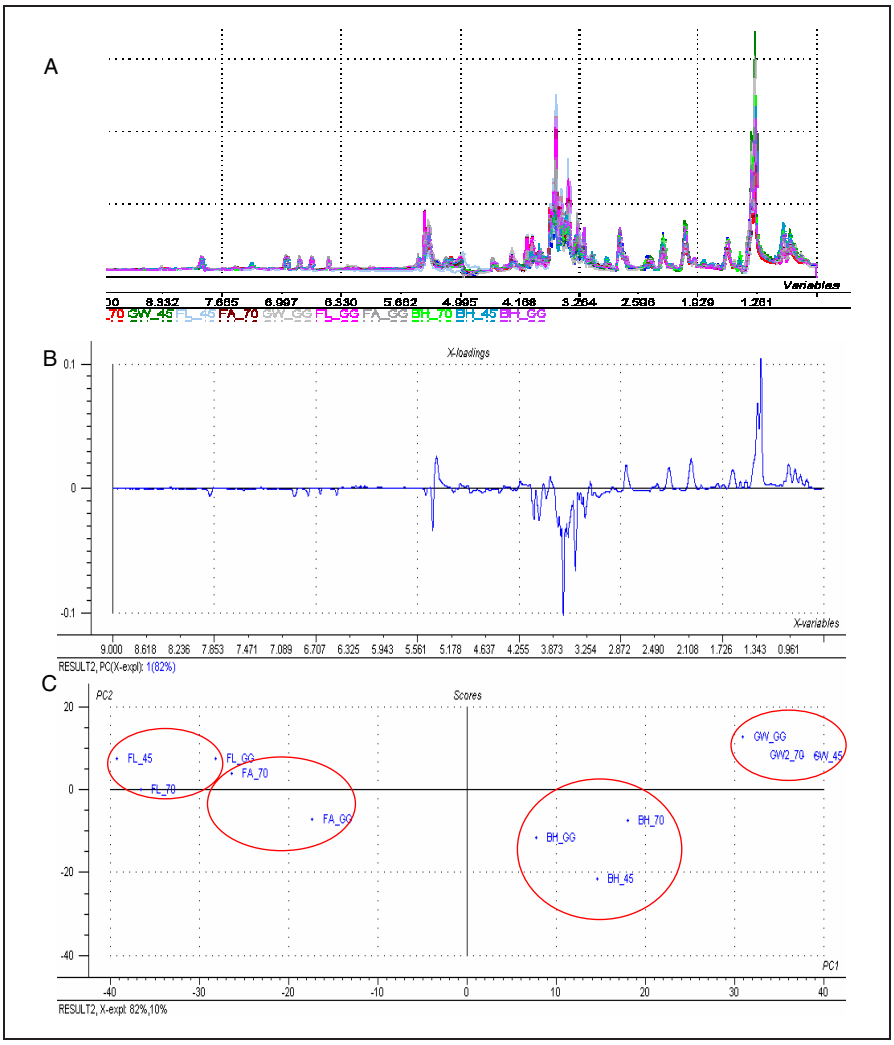


Fig. 2:
Principal component analysis (PCA) of ^{1}H NMR fingerprints of 11 parsley samples (methanol extracts, 400 MHz, methanol-d_4). A: Overlaid ^{1}H NMR spectra; B: loadings of the spectral variables on the first principal component explaining 82% of the variance in the data. C: Scores plot showing the position of each sample in principal component space (second PC plotted vs first PC). 92% of the variance in the data are explained by these two components. The method discriminates well between samples of different origin (clusters highlighted by ellipses) but does *not* distinguish between the drying methods applied (FL, FA, BH, GW: samples of different origin; 45 °C, 70 °C: drying temperature, GG: freeze-dried)

This increase was higher at 70 °C processing temperature as compared to leave material air-dried at 45 °C. Both peaks revealed UV-spectra characteristic for flavones. Using the same field-grown parsley leaves followed by either oven-drying (70 °C) or by lyophilisation a correlation between the increase of peak **1** and decrease of peak **2** during drying process got obvious (Fig. 4). For evaluation of peak identity both peaks were isolated by preparative HPLC from a chlorophyll-free methanolic extract. ESI/MS, 1D- and 2D-NMR spectroscopy confirmed the structures of apiin (**1**, apigenin-7-*O*-apiosyl(1 → 2)glucoside) and 6″-*O*-malonylapiin (**2**, apigenin-7-*O*-apiosyl(1→2)-6″-*O*-malonyl-glucoside). To our knowledge, the complete ^{13}C NMR-data of compound **2** presented herein are published for the first time. Because the ratio of these both compounds seems to be a parameter for differentiation of differently dried or processed parsley samples detailed analysis with mixtures of lyophilised and oven-dried parsley samples was performed. Analysis of a variety of different parsley samples from different origins and processed by different drying-procedures indicated that the ratio of these two compounds indeed could be an appropriate marker to determine the drying process applied to the leave material.

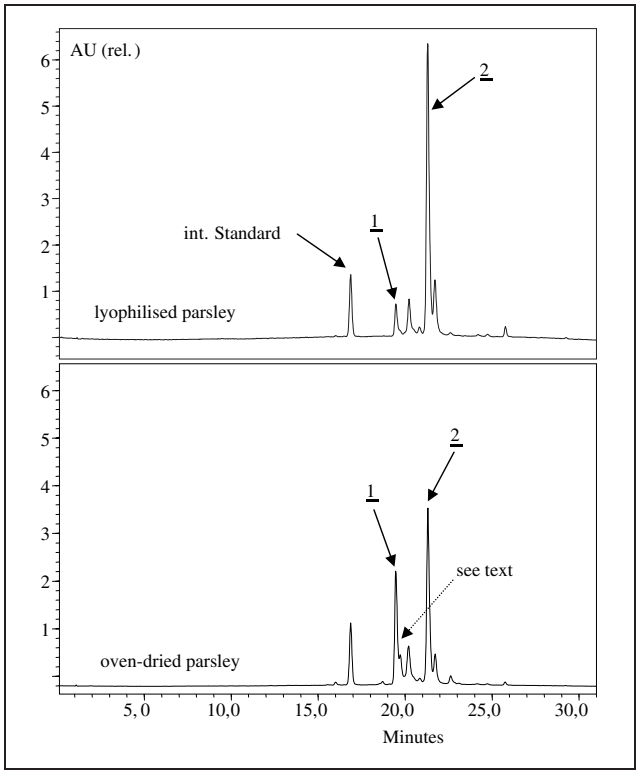


Fig. 3: HPLC of methanolic parsley extract after solid phase extraction ($\lambda = 330$ nm) obtained after different methods of drying (lyophilisation, oven-drying). **1** = apiin, **2** = 6″-*O*-malonylapiin. The y-axis is normalised to the internal standard peak (sinapic acid)

The 6″-*O*-malonylapiin (**2**): apiin (**1**) ratios (mean values) of 4 different origins (A.–D.) were as follows (lyophilised/oven-dried): industrial dried samples: 12.3/1.5; samples from a local market: 4.6/0.5; field-grown parsley: 9.8/0.4; green-house cultivated parsley: 3.6/0.5. These data clearly indicate that in all cases investigated the oven-dried material had a significantly lower malonylapiin:apiin ratio, independently from the origin of the parsley samples.

Figure 5 shows the content of both flavones in lyophilised and oven-dried samples in mixtures with different ratios. During this experiment it became obvious, that by standard calibration also the amount of the respective intermixtures can be calculated.

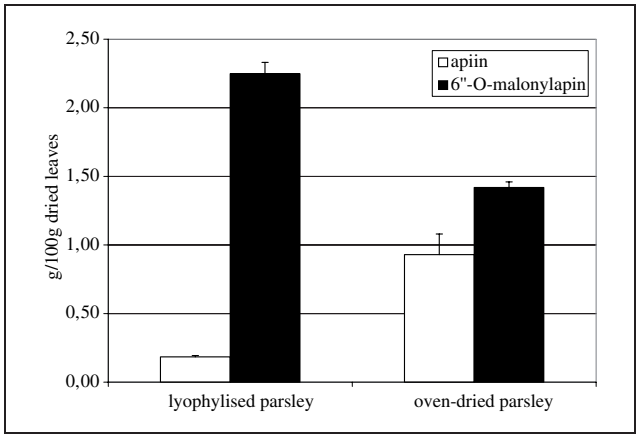


Fig. 4: Changes of contents of apiin and 6″-*O*-malonylapiin (after HPLC-analysis), calculated as sinapic acid, during oven-drying (70 °C) and lyophilisation of field-grown parsley leaves (g/100 g dried plant material). Error bars = mean deviation

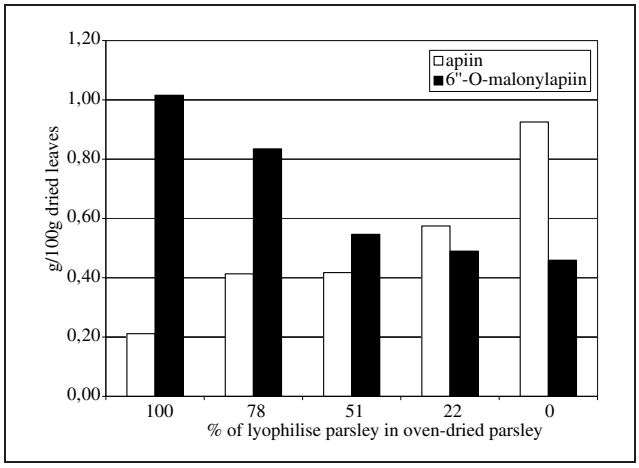


Fig. 5: Contents of apiin (**1**) and 6″-*O*-malonylapiin (**2**) in mixtures of oven-dried (70 °C) and lyophilised parsley leaves

An additional peak occurred in the HPLC chromatogram of oven-dried parsley as a shoulder of peak **1** (Fig. 3). The UV-spectrum indicates a flavone-moiety ($\lambda_{max} = 347$, and 266 nm) but up to now its identity is still unknown – this is under investigation.

3. Discussion

The drying process of plant materials is one of the critical steps during industrial plant processing in order to get stabilised bulk material for further use. Depending on the technology every drying process will lead to more or less changes in sensoric parameters of the material. This can be due to changes in pigmentation and colour; volatile aromatic constituents may be subjected to unspecific evaporation, but also specific temperature-dependent degradation of certain compounds is known; also fermentative changes by cell-associated enzymes are described (Diaz-Maroto et al. 2002; Nüsslein et al. 2000). In the case of parsley, oven-dried leave material tends to discolouration, accompanied by typical changes in aromatic sensation, which can be found in processed parsley extracts. High-quality lyophilised products on the other side are comparable to the fresh material concerning colouration and retainment of sensoric parameters. For an analytical routine differentiation between heat-dried and lyophilised parsley leaves the use of electronic noses is not very effective because the respective odour differences will be below the discrimination level. Detection and analytical determination of volatile trace compounds, responsible for hay-like odour and increasing during a specific drying process was not successful during our investigations. On the other hand, the detection and quantification of 6″-*O*-malonylapiin (**2**) and its degradation product apiin (**1**) in parsley as marker substances for thermal stress seems to be a suitable parameter for a quick and easy routine determination. We assume that the hydrolysis of **2** to **1** is predominantly induced by thermal stress rather than by enzymatic degradation as shown by the increased apiin formation at higher temperature (70 °C) compared to normal fermentative temperature (45 °C). This means that the prediction of the mode of drying, based on the 6″-*O*-malonylapiin/apiin-ratio can only precisely be performed in case the material has been exposed to higher temperatures. The most common process applied to parsley is tunnel drying at 60 to 70 °C, leading to the specific degradation process. Another advantage of the 6″-*O*-malonylapiin/apiin-deter-

mination is seen in the possibility to make a prediction if a mixture of parsley leaves from different drying technologies is analysed. Using standard calibration mixtures, a crude quantification of intermixtures should be possible. The data presented here indicate that the HPLC-determination of the 6″-*O*-malonylapiin/apiin-ratio is a suitable analytical marker for the routine quality control, being more or less independent from the origin of the leave material. Using 4 different parsley qualities from different sources (field-grown, green-house-grown, material from the global market and leaves produced under controlled ecological cultivation) could be differentiated well concerning the respective drying process applied to the leave material. This indicates the specificity, accuracy and robustness of the method.

In addition to this HPLC determination the L*a*b colour measurement can give supporting information on the thermal exposure of the leave material. Especially the brightness values will contribute clearly to a prediction of the applied drying method. On the other hand, values determined by this colour measurement are not exclusively dependent on the mode of drying, but to a minor extent on the cultivation of the parsley. Again, it appears to be an advantage of the malonylapiin/apiin-ratio determination, that the quantification was not significantly influenced by the specific parsley cultivars or culture conditions. Because malonylated flavones are described also for other plants used in food technology (e.g. hot pepper fruits) (Materska et al. 2003) the same monitoring analysis may be applied for analytical control of these spices.

Summarising we conclude that the combination of HPLC determination and L*a*b measurement can provide a valuable tool for prediction of the applied drying method for parsley leaves.

4. Experimental

4.1. Materials

Solvents and reagents were used in analytical quality from Merck, Darmstadt, and Sigma (Germany). Dried parsley leaves (oven-dried and lyophilised) from different origins were obtained from Freeze-Dry Foods GmbH, Greven, Germany. Fresh parsley leaves were from efhi-Frucht-Import, Münster, Germany; the leaves were either lyophilised (Christ Beta 1-16) after freezing in liquid nitrogen or alternatively after freezing at −80 °C; the same material was oven-dried (24 h) at 45 °C or alternatively at 70 °C. Additionally parsley seed material (Mooskrause 2, Bruno Nebelung, Everswinkel, Germany) was cultivated under different conditions (field-grown and greenhouse) at the research agricultural plant of the Institute of Pharmaceutical Biology and Phytochemistry at University of Münster. The fresh material was dried as described above.

4.2. Analytical methods

For microscopy a Zeiss Axioskop (magnification × 100, 400) was used. Determination of volatile oil after steam distillation according European Pharmacopoeia 2005 (20 g leaves, 250 mL water, sampling of oil in 0.5 mL hexane, 2 h distillation time). GC-MS analysis on an Agilent 6890 N gas chromatograph with 5973 mass selective detector; column HP-5MS (30 × 0.25 mm, 0.25 μm film); mobile phase helium, 1 mL/min. Temperature program 70 °C to 120 °C with 3 °C/min, to 250 °C with 8 °C/min. Evaluation by linear retention index (Van den Dool and Kratz 1963) and mass spectra according NIST-spectra library. Internal standards dodecane (Rt < 10 min) and octadecane (Rt > 15 min).

HPLC-analysis of parsley extracts: 50 mg leave material were extracted with 1 mL methanol, containing sinapic acid (216.8 μg/mL, Fluka No. 85429) for 1 min with Ultra-Turrax® homogenisation, followed by centrifugation (1000 × g, 5 min), evaporation of 500 μL to dryness and dissolving in 1.0 mL methanol-water (50 : 50 V/V). The extract was pre-purified by solid phase extraction on a LiChrolut RP18e column (Merck, Darmstadt, Germany): elution with 15 mL methanol-water (50 : 50 V/V), evaporation of the eluate to dryness and dissolving of the residue in 3 mL methanol leads to the test solution. Analysis was done on a waters HPLC system (Waters 717 plus autosampler, Waters 600 controller, in-line degasser AF and Waters 969 PDA; mobile phase A: trifluoroacetic acid (TFA) 0.1% in water, B: methanol. Gradient: 98% A, linear gradient within 30 min to 100% B, 10 min isocratic 100% B. Flow rate 1.3 mL/min. Stationary phase ProSep C18, 150 × 4 mm, 5 μm (Latek, Eppelheim, Germany), λ = 330 nm.

NMR spectra were recorded on a Varian Unity plus 600, Bruker AMX 400 and Bruker ARX 300. All chemical shifts (δ) are given in ppm. For the principal component analysis, 400 MHz spectra of crude methanol extracts (20 mg plant material) were used. Intensity data of the total spectra excluding solvent signals were assigned spectral bins of 0.001 ppm width (total 8196 bins) and submitted to PCA using the program "The Unscrambler", v. 9.2, available from CAMO Process AS, Oslo, Norway.

ESI-MS spectra were recorded on a Quattro LC Waters-Micromass.

L*a*b*-colorimetric measurements were recorded on a Minolta CR-300 colorimeter after calibration against a white reference. Data were recorded as mean values of triplicate measurements of the same sample. Typical STD were smaller than 5% of the given mean value.

Enzymatic activities were determined by ApiZYM® assay (BioMerieux SA, France) according the instruction for the ready-to-use test.

4.3. Isolation and characterisation of 1 and 2

Leaves were ground in liquid N_2. After lyophilisation (35.7 g) and subsequent removal of lipophilic compounds with n-pentane and dichloromethane in a Soxhlet extractor the dried residue was extracted with cold methanol (Ultra-Turrax®). The crude extract was evaporated to dryness (3.4 g) and suspended in H_2O : MeOH (1 : 2). Remaining chlorophyll was removed by chilling (4 °C, 24 h) followed by filtration. An aliquot (0.1 g) of the concentrated filtrate (2.6 g) was dissolved in 10 mL MeOH−H_2O (70 : 30, V/V). Preparative HPLC on a Polaris® RP-8 column (250 × 22.1 mm, 5 μm; linear gradient 0–30 min: MeOH 70% → 100%) provided 5 fractions; F1 (10.5–13.5 min): 48.9 mg, F2 (13.5–16.0 min): 3.2 mg, F3 (16.0–18.0 min): 2.7 mg, F4 (18.0–25.0 min): 7.4 mg and F5 (25.0–30.0 min): 0.9 mg. F2 (containing **1**) and F4 (containing **2**) were used for structure verification.

4.3.1. Apiin (1)

^{1}H NMR data were consistent with published data (Eckey-Kaltenbach et al. 1983; Markham and Geiger 1994). ESI-MS (nanospray): ESI pos. mode: $[M + H]^+ = 565.3$ m/z ; ESI neg. mode: $[M + Cl]^- = 599.3$ m/z, $[M - H]^- = 563.3$ m/z.

4.3.2. 6″-O-Malonylapiin (2)

The ^{1}H NMR data were almost in complete consistency with those of **1**; particular variations: shifts of signals due to protons H-6″A/B (+0.6 ppm in MeOH-d_4) and H-6 (−0.05 ppm in MeOH-d_4). We observed a complete exchange of the acidic methylene protons (H-2^{IV}) in MeOH-d_4, thus further measurements were performed in DMSO-d_6. Assignments were confirmed by 2D-NMR experiments (COSY, g-HMBC, g-HSQC).

^{1}H NMR (DMSO-d_6, 600 MHz, δ 2.490): δ 7.95 (d, J = 8.8 Hz, H-2′/6′), δ 6.93 (d, J = 8.8 Hz, H-3′/5′), δ 6.86 (s, H-3), δ 6.77 (d, J = 2.2 Hz, H-8), δ 6.46 (d, J = 2.2 Hz, H-6), δ 5.34 (d, J = 1.3 Hz, H-1‴), δ 5.20 (d, J = 7.5 Hz, H-1″), δ 4.38 (dd, J = 12.0/1.7, H-6″A), δ 4.11 (dd, J = 12.0/7.0, H-6″B), δ 3.91 (d, J = 9.5, H-4‴A), δ 3.78 (m, H-4″), δ 3.74 (br s, H-2‴), δ 3.66 (d, J = 9.5 Hz, H-4‴B), δ 3.54 (dd, H-2″), δ 3.50 (m, H-3″), δ 3.39 (H-2^{IV}), δ 3.28 (s, H-5‴), δ 3.21 (m, H-5″); ^{13}C NMR (DMSO-d_6, 125 MHz, δ 39.50): δ 182.0 (C-4), 167.8 (3^{IV}), 166.8 (C-1^{IV}), 164.3 (C-2), 162.4 (C-7), 161.3 (C-4′), 161.1 (C-5), 156.9 (C-9), 128.6 (C-2′/6′), 121.0 (C-1′), 116.0 (C-3′/5′), 108.7 (C-1‴), 105.4 (C-10), 103.1 (C-3), 99.4 (C-6), 97.8 (C-1″), 94.7 (C-8), 79.3 (C-3‴), 76.4 (C-3″), 76.0 (C-2‴), 75.5 (C-2″), 74.0 (C-4‴), 73.6 (C-4″), 69.8 (C-5″), 64.1 (C-6″), 63.9 (C-5‴), 41.4 (C-2^{IV}).

ESI-MS (nanospray): ESI pos. mode $[M + H]^+ = 651.3$ m/z, $[M + Na]^+ = 673.3$ m/z; ESI neg. mode $[M - H]^- = 649.3$ m/z.

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References

Bolkent S, Yanadag R, Ozsoy-Sacan O, Karabulut-Bulan O (2004) Effects of parsley (*Petroselinum crispum*) on the liver of diabetic rats: a morphological and biochemical study. Phytother Res 18: 996–999.

Diaz-Maroto MC, Perez-Coello MS, Cabezudo MD (2002) Effect of different drying methods on the volatile components of parsley (*Petroselinum crispum* L.). Eur Food Res Technol 215: 227–230.

Eckey-Kaltenbach H, Heller W, Sonnenbichler J, Zetl I, Schäfer W, Ernst D, Sandermann H (1983) Oxidative stress and plant secondary metabolism: 6-O-malonylapiin in parsley. Phytochem 34: 687–691.

Hänsel R, Keller K, Rimpler H, Schneider G (ed.) (1994) *Petroselinum*. In Hagers Handbuch der Pharmazeutischen Praxis, vol. 6, pp. 105–115.

Luthria DL, Mukhopadhyay S, Kwansa AL (2006) A systematic approach for extraction of phenolic compounds using parsleys (*Petroselinum crispum*) flakes as a model substrate. J Sci Food Agric 86: 1350–1358.

Markham KR, Geiger H (1994) in: Harborne JB (ed) The Flavonoids, Advances in Research since 1986, Chapman & Hall, pp. 441–497.

Masanetz C, Grosch W (1998) Hay-like flavour of dry parsley. Z Lebensm Unters Forsch A 206: 114–220.

Materska M, Piacente S, Stochmal A, Pizza C, Oleszek W, Perucka I (2003) Isolation and structure elucidication of flavonoid and phenolic acid glycosides from pericarp of hot pepper fruit *Capsicum anuum* L. Phytochem 63: 893–898.

Meyer H, Bolarinwas A, Wolfram G, Linseisen J (2006) Bioavailability of apigenin from apiin-rich parsley in humans. Ann Nutr Metab 50: 167–172.

Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knutsen P, Sandstrom B, Dragsted LO (1999) Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. Br J Nutr 81: 425–426.

Nüsslein B, Kurzmann M, Bauer R, Kreis W (2000) Enzymatic degradation of cichoric acid in *Echinacea purpurea* preparations. J Nat Prod 63: 1615–1618.

Ozsoy-Sacan O, Yanardag R, Orak H, Ozgey Y, Yarat A, Tunali T (2006) Effects of parsley (*Petroslinum crispum*) extract versus glibornuride on the liver of streptozotocin-induced diabetic rats. J Ethnopharmacol 104: 175–181.

Simon EJ, Quinn J (1988) Characterisation of essential oils of parsley. J Agric Food Chem 36: 467–472.

Van den Dool H, Kratz PD (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr 11: 463–471.

Yanardag R, Bolkent S, Tabakoglu-Oguz A, Ozsoy-Sacan O (2003) Effects of *Petroselinum crispum* extract on pancreatic B cells and blood glucose of streptozotocin-induced diabetic rats. Biol Pharm Bull 26: 1206–1210.

Yoshikawa M, Uemura T, Shimoda H, Kishi A, Kawahara Y, Matsuda H (2000) Phytoestrogens from the aerial part of *Petroselinum crispum* Mill. (Parsley) and structure of 6-acetylapiin and a new monoterpene glycoside, petroside. Chem Pharm Bull 48: 1039–1044.

Zidorn C, Johrer C, Ganzera M, Schubert B, Sigmund EM, Mader J, Greil R, Ellmerer EP, Stuppner H (2005) Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley and parsnip and their cytotoxic activities. J Agric Food Chem 53: 2518–2523.