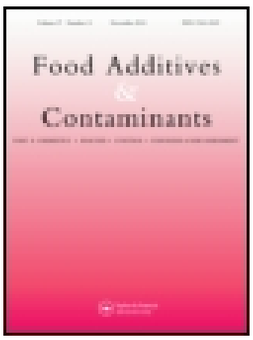

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Residual solvent or intrinsically formed during production: analysing volatile compounds in unrefined vegetable oils using headspace gas chromatography coupled with mass spectrometry

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ABSTRACT

Vegetable oils are an essential part of a healthy and balanced diet and have major economic significance. The type of production, whether performed by extraction with solvents, or by mechanical techniques, significantly influences the quality of the oil and its potential field of use. Occasionally, volatile organic substances, which are considered indicative for an oil that has been produced by solvent extraction, are detected in unrefined vegetable oils. This can have a negative impact on high-quality oil mills and their reputation. The goal of the study was to analyse unrefined oils of different raw materials for such compounds. A previously developed and validated method using headspace gas chromatography coupled with a mass spectrometry was used for this task. Another aim was to determine the origin of any solvent residues in the vegetable oils and to find ways to avoid them. Complementary measurements by solid phase micro extraction gas chromatography were conducted to compare the results of the measurements to an orthogonal methodology. Multivariate data analysis was used to find correlations between the spectrum of substances in the oil and other parameters like the producer of the oil or the pattern of fatty acids.

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Unrefined vegetable oil; volatiles; solvent residue; headspace-gas chromatography; mass spectrometry; triglyceride degradation

Introduction

The production of unrefined vegetable oils has a long tradition in southern Styria which is a region in the south of Austria. Styrian pumpkin seed oil is well known as a local specialty and has economical relevance for the region. It was registered as a "Protected Geographical Indication" item (PGI) in the European Union's (EU) Database Of Origin & Registration (DOOR) in 1996 (European Commission 1996). However, other high-quality oils from different oil seeds are produced in the region as well, like linseed oil, hazelnut oil and walnut oil. In contrast to animal fats, vegetable oils have a high content of unsaturated fatty acids and other essential nutritious components, like fat-soluble vitamins and antioxidants. Due to these valuable ingrediencies, they play an important role in a balanced healthy diet. (Timmermann 1990). The choice of the oil production technique is a first indication of the quality of the final product. Oils can be produced either by mechanical techniques or by extraction with organic solvents (Krist 2013). The pressing of oilseeds is the traditional technique in Styria. The produced oil is used for high-quality foods or direct consumption. Most of the flavouring substances and other ingredients are preserved and a valuable product can be achieved regarding both its nutritional as well as its sensory parameters. The roasting of the crushed oil seeds prior to the pressing process enables the separation of the oil from other parts of the plant, as well as the formation of substances which are essential for the typical flavour (Siegmund and Murkovic 2004). For the production of pumpkin seed oil, this process employs temperatures of approximately 130°C and can take up to 60 minutes (Poehlmann and Schieberle 2013). In contrast to mechanical processes, extraction with solvents is a more cost-effective technique, and is therefore used in the production of oils which are produced in high volume for the food industry as well as

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other industrial applications (Baltes and Matissek 2011). The complete removal of these solvents from the produced oil can be a challenge. Even small traces of solvents can be detected with modern analytic techniques. (Kumar and Gow 1994). Investigating oil samples for volatile, organic substances is therefore a common method for determining whether an oil was extracted or produced by mechanical means (Michulec and Wardencki 2004). Oil mills, which are confronted with the accusation of producing their oil via extraction of the oilseeds, face substantial economical and reputational damage. Several studies have already investigated the influence of the roasting process of oil seeds, on the formation of volatile substances. However, the focus is usually put on flavouring substances instead of compounds which are also used as solvents (Ozel et al. 2014; Gracka et al. 2016; Zhang et al. 2016). The topic of contamination of edible oils with mineral oils and solvent residues has significantly gained in importance over recent years, especially in Europe. Since a variety of hydrocarbons can be found naturally in unrefined vegetable oils, it is often not easy to distinguish between natural occurrence and contamination (Moreda et al. 2001). Many of these compounds can be attributed to fat degradation which can be promoted by the influence of light and heat (Frankel 1983, 2005; Angelo 1992; Kamal-Eldin 2003; Choe and Min 2006). In addition to technical challenges, producers are faced with the consumer's wish of obtaining a product which is without impurities, but also has been produced under natural and organic conditions. The oil's tendency to absorb compounds from the environment further complicates the matter (Belitz et al. 1982). Existing EU law regulates contaminants (e.g. polycyclic aromatic hydrocarbons, PAH) in edible oils as well as recommends monitoring practices (European Commission 2011, 2017). However, not all occurring compounds are regulated within EU law as limits for solvent residues apply only for oils which have been declared to be extracted with solvents. (European Parliament & European Union 2009). With analytical instrumentation and methodology becoming increasingly sensitive, even the smallest amounts of volatiles and other substances can be detected in vegetable oil. This leads to unclear situations, especially if the legal requirements are not explicitly defined. Producers who are faced with analytical reports, claiming the presence of minor residues of solvents in their unrefined oils, have no legislative leeway if food traders reject their products. Choosing a sensible and careful approach to this issue, is critical for producers of unrefined oils, food traders, as well as for analytical chemists. Lawmakers must be aware that there is need of addressing these problems and avoiding legislative uncertainty concerning the occurrence of solvent residues in unrefined vegetable oils.

The aim of the study was, to analyse oils which demonstrably were produced by pressing of the oilseeds, for volatile, organic substances, which could possibly be associated with solvent extraction. The producers of the oils had previously been confronted with analysis reports explicitly categorising detected volatile compounds like 2-butanone and toluene as solvent residues. The analysis of oils of different producers and from a variety of oilseeds was conducted using gas chromatography (GC) coupled with a single quadrupole mass spectrometer (MS) and static headspace (HS) injection. Selected samples were heat-treated in a thermal conditioning experiment, which would provide information about the influence of heat on the formation of these volatile substances. The focus was therefore put on volatile substances, which are specifically used as solvents, and not on the whole spectrum of volatile flavouring compounds.

Materials and methods

Analytes

Standard materials for the following analytes were purchased for the method development as well as for the quantitative evaluation: 1,1,1,2-tetrachloroethane (abcr GmbH, Karlsruhe, Germany), 1,1,2-trichloroethane (Acros Organics, Geel, Belgium), bromodichloromethane, dibromochloromethane, styrene, trichloroethene (Alfa Aesar, Karlsruhe, Germany), chloroform (Chem-Lab, Zedelgem, Belgium), ethylbenzene (Lactan, Graz, Austria), 1,1-dichloroethane, 2-butanone, acetone, benzene, dichloromethane, ethyl acetate, heptane, hexane, pentane, toluene (Roth, Karlsruhe, Germany), 1,1,1-trichloroethane,

1,2-dichloroethane, tribromomethane, cis-1,2-dichloroethene, m-xylene, p-xylene, o-xylene, tetrachloroethene, tetrachloromethane and trans-1,2-dichloroethene (Sigma&Aldrich, Steinheim, Germany). Benzene-D_6, acetone-D_6 and chloro form-D_1, which were used as internal standards, were also purchased from Roth (Karlruhe, Germany).

Method and equipment

The method used in this study was based on EN ISO 16035:2005 (Österreichisches Normungsinsitut 2005) and modified regarding the analytical scope. However, other studies regarding methods for solvent residue analysis have already been published (Kumar and Gow 1994; Michulec and Wardencki 2004, 2005) and were used as an orientation in some regards of the method development. The instrument assembly consisted of an HP 6890 coupled with an Agilent Technologies 5973 network MSD, while the sampling was done with an HP G1290A Headspace-Sampler. The capillary column used for all analysis was an Agilent Technologies DB-624, 30 m x 0.25 mm, 1.40 µm film. Helium 5.0 was used as carrier gas and exerted a constant column pressure of 10 psi (68.95 kPa), while the sample inlet was held at 250°C. The average column flow velocity was 40 cm sec^{-1}. The GC oven was held at 50°C for four minutes (i), then heated at 8°C min^{-1} to 100°C, which was held for five minutes (ii) and lastly heated at 20°C min^{-1} to 200°C, which was held for one minute (iii). This resulted into a total runtime of 21 minutes and 15 seconds. The headspace sampler enables static headspace analysis. The transfer line to the mass spectrometer was heated to 290°C, the electron ionisation source to 230°C and the quadrupole to 150°C. Since the volatile analytes mainly occur in low concentrations, they were measured using the SIM (Selected Ion Monitoring) mode to enhance the sensitivity. For every analyte in the method, one quantifier and at least two qualifiers were added (except acetone, which only has two measurable ions). Table 1 shows the resulting retention time as well as the m/z-values for every analyte. The method was validated comprehensively by performing spiking experiments using pumpkin seed oil (seven concentration levels with four parallel measurements each). Method-relevant parameters like the limits of detection (LOD) and quantitation (LOQ), were calculated using this validation (calibration method, $P < .05$). The limit of detection (LOD) and the limit of quantitation (LOQ) of all compounds can be seen in Table 2. The statistical software tool used for the multivariate data analysis was Mass Profiler Professional Version 14.9.1 (Agilent Technologies Inc. and Strand Life Sciences Pvt. Ltd.).

Table 1. Chromatographic and mass spectrometric method parameters (deuterated compounds are labelled by -D_n).

Substance	RT (min)	m/z 1	m/z 2	m/z 3
Pentane	2.355	57	43	72
Acetone-D_6	2.788	64	46	
Acetone	2.835	43	58	
Dichloromethane	3.268	84	86	88
(E)-1,2-Dichloroethene	3.575	96	98	63
Hexane	3.883	86	57	71
1,1-Dichloroethane	4.103	63	98	83
(Z)-1,2-Dichloroethene	4.907	96	98	63
2-Butanone	4.938	72	57	43
Ethyl acetate	5.007	88	70	73
Chloroform-D_1	5.338	84	86	119
Chloroform	5.368	85	83	118
1,1,1-Trichloroethane	5.633	97	119	99
Tetrachloromethane	5.872	119	84	117
Benzene-D_6	6.136	56	82	81
Benzene	6.200	78	77	52
1,2-Dichloroethane	6.248	98	100	62
Heptane	6.600	100	71	70
Trichloroethene	7.231	130	132	95
Bromo(dichloro)methane	8.078	83	127	129
Toluene	9.418	91	92	93
1,1,2-Trichloroethane	10.188	97	99	132
Tetrachloroethene	10.389	166	168	129
Dibromo(chloro)methane	10.920	127	210	129
1,1,1,2-Tetrachloroethane	12.508	117	131	119
Ethylbenzene	12.580	106	91	65
m-Xylene and p-xylene	12.919	106	105	91
o-Xylene	14.095	91	105	106
Styrene	14.166	104	103	78
Bromoform	14.743	173	252	171

Standard solutions and calibration

For preparing a stock solution, the pure substances were diluted in methanol. The added volumes were based on the LOD and on the expected concentration of the corresponding analyte in most samples. These estimates had been established after preliminary sample measurements. The solution was further diluted and mixed with the internal standard to prepare the individual calibration standard solutions. Highly refined olive oil (Sigma-Aldrich Handels GmbH; Vienna, Austria) was used as a matrix material for the preparation of the calibration standards, because it contained negligible amounts of volatile substances (≪ LOD). For

Table 2. List of analysed compounds and their calculated LOD and LOQ (calibration method, seven concentration levels, four repetitions each, $P < .05$) RSD% and the coefficient of determination were calculated from a series of calibrations (four calibrations, five levels).

Substance	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	RSD %	Coefficient of determination
Pentane	0.1	0.3	4%	0.9996
Acetone	0.3	1	10%	0.9878
Dichloromethane	0.03	0.09	9%	0.9984
(E)-1,2-Dichloroethene	0.003	0.01	5%	0.9998
Hexane	0.01	0.03	8%	0.9997
1,1-Dichloroethane	0.003	0.01	4%	0.9998
(Z)-1,2-Dichloroethene	0.003	0.01	3%	0.9998
2-Butanone	0.03	0.1	2%	0.9995
Ethyl acetate	0.1	0.3	2%	0.9997
Chloroform	0.01	0.03	5%	0.9987
1,1,1-Trichloroethane	0.001	0.003	4%	0.9986
Tetrachloromethane	0.003	0.01	4%	0.9999
Benzene	0.003	0.01	3%	0.9999
1,2-Dichloroethane	0.003	0.01	4%	0.9979
Heptane	0.01	0.03	3%	0.9941
Trichloroethene	0.003	0.01	2%	0.9979
Bromo(dichloro)methane	0.003	0.01	2%	0.9997
Toluene	0.003	0.01	2%	0.9999
1,1,2-Trichloroethane	0.003	0.01	2%	0.9997
Tetrachloroethene	0.003	0.01	2%	0.9995
Dibromo(chloro)methane	0.003	0.01	2%	0.9994
1,1,1,2-Tetrachloroethane	0.003	0.01	2%	0.9989
Ethylbenzene	0.003	0.01	3%	0.9999
m-Xylene and p-xylene	0.003	0.01	4%	0.9998
o-Xylene	0.003	0.01	3%	0.9976
Styrene	0.003	0.01	2%	0.9996
Bromoform	0.003	0.01	2%	0.9999

every calibration standard, 10.00 g of this oil were spiked with 100 µl of the corresponding calibration solution. The calibration consisted of five levels with an additional blank and was prepared fresh for each measurement.

Samples

A total of 69 unrefined vegetable oil were analysed using the described method. The samples were divided into two sets which were drawn six months apart. Overall 58 pumpkin seed oils, 3 linseed oils, 3 walnut oils, 2 poppy seed oils, a hazelnut oil, a hemp seed oil and a salad oil, which is a mixture of pumpkin seed oil and rapeseed oil, were measured.

Sample preparation

An aliquot of 10.00 g of each sample was weighed into a 20 ml headspace vial and 100 µl of a methanol solution, which contained the internal standard were added. The vial was closed with an aluminium crimp cap equipped with Teflon-coated butyl rubber septum. A volume of 10.00 g of the highly refined olive oil, also mixed with 100 µl of the internal standard solution, were used as blank. The prepared samples were put into the analytic system.

Thermal conditioning experiment

To investigate the influence of temperature on oilseeds and oil during the roasting process, samples of different oils and also a sample of finely ground pumpkin seeds were heated to 120°C for 24 hours. After 2 hours, 4 hours, 8 hours, 16 hours and 24 hours, samples were drawn. All the samples were left to cool down to room temperature before they were measured.

Determination of the fatty acid spectrum

The fatty acid spectra of all six oils which were subject to the thermal conditioning, were determined by an independent and accredited laboratory.

Comparative measurements using solid phase micro extraction GC-MS

The results of the HS-GC-MS method which was developed for this project, were compared to the measurements of a complementary system – Solid Phase Micro Extraction (SPME) GC-MS. In contrast to all the other analyses, these measurements took place at the Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology. The instrument was equipped with a cryogenic cooling system for the GC oven which allowed for low initial temperatures. The aim of the comparative analyses was to verify the results using a different sample injection system, which would impose less thermal stress on the samples. For the measurements, 1 g of a pumpkin seed oil was weighed into a 20 ml-SPME vial, a glass coated stirring bar was added, and the vial closed using a magnetic crimp cap with a Teflon-coated rubber septum. A rudimentary two-point sample addition experiment was prepared to calculate the concentrations of any analytes found in the sample.

Results and discussion

HS-GC-MS measurements of the oil samples

In every sample of the 69 analysed oils, at least small residues of volatile substances, which are commonly used as solvents, were found. No halogenated compounds were detected in the analysed oils. The substances which occurred most frequently and in the highest concentrations were acetone, 2-butanone, pentane (0.11–1.9 mg kg^{-1}), as well as toluene, heptane, ethyl acetate and styrene (0.014–0.40 mg kg^{-1}) (4 replicates, RSD: <15 %). Detailed results are listed in Table 3. Since the examined oil samples were produced by pressing the oilseeds and not by extracting them, the findings cannot be traced back to solvent residues. Pentane in particular can be used as an indicator for oxidative degradation of vegetable oils (Pinnel and Vandegans 1996), which leads to the assumption that the other substances might have a similar origin.

Comparative measurements using SPME-GC-MS

The complementary measurements of the pumpkin seed oil using SPME-GC-MS showed clearly that the substances and their concentration ranges compared well to prior results obtained with the HS-GC-MS system. Table 4 shows the results of the SPME-GC-MS measurement in detail. The findings are typical for pumpkin seed oil based on the HS-GC-MS analysis. Pentane was detected in higher concentrations compared to other typical pumpkin seed oils. This could be explained by oxidation of the oil sample. Since the spiking levels were set before the measurement, some of the concentrations were not optimal for several of the analytes. Too high or too low spiking concentrations increase the standard deviations of the quantitative analysis.

Thermal conditioning experiment

Initial quantitative evaluations of the sample measurements indicated a connection of the abundance of certain analytes like 2-butanone and pentane in the sample and the corresponding oil producer. A conditioning experiment was conducted to investigate the assumption, that the oil production process is linked with the occurrence of substances, which are commonly used as solvents (3 replicates, RSD: <20 %). A comparison of the total ion current (TIC) chromatograms of the different measurements already showed increasing signal intensities of some of the compounds over time (Figure 1). Some of the signals in the chromatogram were not part of the method's scope but responded to the same SIM parameters. An additional scanning measurement was performed and a mass spectral library (NIST/EPA/NIH Mass Spectral Library 05) was searched for the unknown signals. The compounds which were identified with the highest match score (>95%) were ethanol, propanol, butanal, (E)-oct-2-ene, piperidine and hexanal. All these substances are known to occur naturally in vegetable oils and some are responsible for the flavour of the oil (deMan 1999). To illustrate the data, the signal areas of the substances were plotted against time, which can be seen in Figure 2 for the compounds which were in the initial scope of the analytical method and in Figure 3 for the compounds which were identified by the database search. Each oil was produced from a different oilseed (hemp seed oil, hazelnut oil, pumpkin seed oil, linseed oil, poppy seed oil, walnut oil). A sample of ground pumpkin seeds was investigated in this experiment as well.

It became evident that the concentration of the measured substances in the oils increased over the course of the conditioning experiment. The extent of the concentration increase depended on the compound as well as on the oil type. Examples of this can be seen in the increasing concentrations of m-xylene, p-xylene (Figure 2(d)) and toluene (Figure 2(h)) in pumpkin seeds and pumpkin seed oil as well as the content of heptane (Figure 2(e)) and hexane (Figure 2(f)) in hazelnut oil. Even though the concentration of linear alkanes increased in every sample with prolonged thermal stress, the rate was still dependent on the oil variety. Pumpkin seed oil generally seemed to behave differently under thermal stress than the other tested oil varieties. This is likely due to the significantly higher content in colouring substances like chlorophylls which also act as sensitizers for the oxidation of the triglycerides (Choe and Min 2006). In contrast to the vegetable oils,

Table 3. Results of the measurements of sample sets one (1–32) and two (33–69) which were obtained and analysed six months apart (three repetitions per sample).

sample	n-pentane (mg kg^{-1})	acetone (mg kg^{-1})	2-butanone (mg kg^{-1})	ethyl acetate (mg kg^{-1})	n-heptane (mg kg^{-1})	toluene (mg kg^{-1})	ethyl benzene (mg kg^{-1})	m-/p-xylene (mg kg^{-1})	styrene (mg kg^{-1})
1 poppy	<0.30	n.d.	<0.10	n.d.	n.d.	0.013 ± 0.001	n.d.	<0.010	n.d.
2 pumpkin	0.34 ± 0.02	1.6 ± 0.2	0.96 ± 0.03	n.d.	n.d.	0.061 ± 0.004	n.d.	0.025 ± 0.002	0.014 ± 0.001
3 pumpkin	0.84 ± 0.02	2.2 ± 0.1	1.3 ± 0.1	n.d.	n.d.	0.055 ± 0.001	n.d.	0.015 ± 0.001	0.014 ± 0.001
4 walnut	1.1 ± 0.1	<1.0	0.24 ± 0.01	n.d.	<0.03	0.023 ± 0.001	n.d.	<0.010	0.022 ± 0.001
5 linseed	n.d.	<1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6 pumpkin	n.d.	1.4 ± 0.2	0.44 ± 0.02	n.d.	n.d.	0.028 ± 0.001	n.d.	n.d.	n.d.
7 pumpkin	<0.30	1.8 ± 0.2	0.85 ± 0.03	n.d.	n.d.	0.055 ± 0.001	n.d.	n.d.	n.d.
8 pumpkin	<0.30	1.4 ± 0.1	0.68 ± 0.03	n.d.	n.d.	0.031 ± 0.001	n.d.	n.d.	n.d.
9 pumpkin	<0.30	1.8 ± 0.1	0.83 ± 0.03	n.d.	n.d.	0.033 ± 0.001	n.d.	n.d.	n.d.
10 pumpkin	0.72 ± 0.04	1.4 ± 0.1	0.64 ± 0.04	n.d.	n.d.	0.080 ± 0.007	n.d.	0.035 ± 0.006	<0.010
11 pumpkin	0.54 ± 0.05	1.4 ± 0.1	0.69 ± 0.02	n.d.	0.04 ± 0.01	0.056 ± 0.001	n.d.	<0.010	<0.010
12 pumpkin	0.33 ± 0.01	1.4 ± 0.1	0.65 ± 0.02	n.d.	n.d.	0.054 ± 0.001	n.d.	n.d.	n.d.
13 pumpkin	0.33 ± 0.01	1.4 ± 0.1	0.67 ± 0.02	n.d.	n.d.	0.061 ± 0.001	n.d.	n.d.	n.d.
14 pumpkin	0.46 ± 0.02	1.8 ± 0.1	1.1 ± 0.1	n.d.	n.d.	0.059 ± 0.002	n.d.	n.d.	<0.010
15 pumpkin	0.70 ± 0.03	1.9 ± 0.1	0.81 ± 0.03	n.d.	<0.03	0.050 ± 0.001	n.d.	n.d.	<0.010
16 pumpkin	0.32 ± 0.02	1.6 ± 0.1	0.79 ± 0.02	n.d.	n.d.	0.040 ± 0.001	n.d.	n.d.	<0.010
17 pumpkin	n.d.	<1.0	0.20 ± 0.01	n.d.	n.d.	0.021 ± 0.001	n.d.	n.d.	n.d.
18 pumpkin	0.34 ± 0.02	1.2 ± 0.1	0.48 ± 0.02	n.d.	n.d.	0.049 ± 0.001	n.d.	n.d.	<0.010
19 pumpkin	n.d.	1.5 ± 0.1	0.55 ± 0.02	n.d.	n.d.	0.029 ± 0.001	n.d.	n.d.	<0.010
20 pumpkin	n.d.	1.4 ± 0.1	0.24 ± 0.01	n.d.	n.d.	0.033 ± 0.001	n.d.	n.d.	n.d.
21 pumpkin	<0.30	<1.0	0.19 ± 0.01	n.d.	n.d.	0.015 ± 0.001	n.d.	n.d.	n.d.
22 pumpkin	n.d.	<1.0	0.36 ± 0.01	n.d.	n.d.	0.017 ± 0.001	n.d.	n.d.	n.d.
23 pumpkin	n.d.	<1.0	0.48 ± 0.02	n.d.	n.d.	0.020 ± 0.001	n.d.	<0.010	<0.010
24 pumpkin	n.d.	<1.0	0.21 ± 0.01	n.d.	n.d.	0.021 ± 0.001	n.d.	n.d.	n.d.
25 pumpkin	<0.30	<1.0	0.29 ± 0.01	n.d.	n.d.	0.021 ± 0.001	n.d.	n.d.	n.d.
26 pumpkin	<0.30	<1.0	0.26 ± 0.01	n.d.	n.d.	0.019 ± 0.001	n.d.	n.d.	n.d.
27 pumpkin	<0.30	<1.0	0.29 ± 0.02	n.d.	n.d.	0.022 ± 0.001	n.d.	n.d.	n.d.
28 pumpkin	n.d.	<1.0	0.25 ± 0.01	n.d.	n.d.	0.025 ± 0.001	n.d.	n.d.	n.d.
29 pumpkin	0.50 ± 0.04	1.3 ± 0.1	0.62 ± 0.04	n.d.	n.d.	0.041 ± 0.001	n.d.	n.d.	<0.010
30 pumpkin	0.62 ± 0.05	1.2 ± 0.1	0.46 ± 0.03	n.d.	n.d.	0.041 ± 0.002	n.d.	n.d.	n.d.
31 pumpkin	0.50 ± 0.03	1.2 ± 0.1	0.53 ± 0.03	n.d.	n.d.	0.037 ± 0.001	n.d.	n.d.	<0.010
32 pumpkin	n.d.	<1.0	0.15 ± 0.01	n.d.	n.d.	0.020 ± 0.001	n.d.	n.d.	n.d.
33 pumpkin	0.59 ± 0.05	1.2 ± 0.08	0.45 ± 0.03	n.d.	<0.03	0.051 ± 0.002	0.017 ± 0.001	0.074 ± 0.004	<0.01
34 pumpkin	0.37 ± 0.02	1.2 ± 0.09	0.51 ± 0.04	n.d.	n.d.	0.037 ± 0.001	n.d.	<0.01	<0.01
35 pumpkin	0.69 ± 0.06	1.5 ± 0.01	0.69 ± 0.05	n.d.	<0.03	0.068 ± 0.003	n.d.	<0.01	<0.01
36 pumpkin	1.0 ± 0.05	1.5 ± 0.02	0.57 ± 0.02	n.d.	<0.03	0.041 ± 0.002	n.d.	n.d.	<0.01
37 pumpkin	0.56 ± 0.05	1.4 ± 0.01	0.6 ± 0.01	n.d.	<0.03	0.043 ± 0.001	n.d.	n.d.	<0.01
38 pumpkin	0.48 ± 0.02	1.4 ± 0.01	0.64 ± 0.04	n.d.	n.d.	0.062 ± 0.002	n.d.	<0.01	<0.01
39 pumpkin	0.60 ± 0.03	1.3 ± 0.01	0.46 ± 0.02	n.d.	<0.03	0.041 ± 0.001	n.d.	n.d.	<0.01
40 poppy	0.44 ± 0.02	<1.0	<0.1	n.d.	<0.03	<0.01	n.d.	n.d.	n.d.
41 walnut	1.3 ± 0.09	<1.0	0.25 ± 0.03	n.d.	<0.03	0.021 ± 0.001	n.d.	<0.01	0.016 ± 0.001
42 pumpkin	n.d.	<1.0	n.d.	0.40 ± 0.02	n.d.	<0.01	n.d.	n.d.	n.d.
43 linseed	n.d.	<1.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
44 pumpkin	1.3 ± 0.1	1.3 ± 0.08	0.74 ± 0.05	n.d.	0.047 ± 0.009	0.065 ± 0.004	n.d.	0.023 ± 0.002	0.015 ± 0.001
45 pumpkin	1.6 ± 0.1	1.2 ± 0.09	0.70 ± 0.05	n.d.	<0.03	0.058 ± 0.003	n.d.	0.011 ± 0.001	0.014 ± 0.001
46 pumpkin	<0.3	1.9 ± 0.2	0.81 ± 0.02	n.d.	n.d.	0.029 ± 0.001	n.d.	n.d.	<0.01
47 pumpkin	<0.3	1.6 ± 0.07	0.58 ± 0.05	n.d.	n.d.	0.029 ± 0.001	n.d.	n.d.	<0.01
48 pumpkin	0.40 ± 0.04	1.6 ± 0.03	0.76 ± 0.07	n.d.	n.d.	0.035 ± 0.001	n.d.	n.d.	<0.01

(*Continued*)

Table 3. (Continued).

sample	n-pentane (mg kg^{-1})	acetone (mg kg^{-1})	2-butanone (mg kg^{-1})	ethyl acetate (mg kg^{-1})	n-heptane (mg kg^{-1})	toluene (mg kg^{-1})	ethyl benzene (mg kg^{-1})	m-/p-xylene (mg kg^{-1})	styrene (mg kg^{-1})
49 pumpkin	0.35 ± 0.01	1.4 ± 0.01	0.49 ± 0.01	n.d.	n.d.	0.046 ± 0.002	n.d.	n.d.	<0.01
50 hemp	0.56 ± 0.05	<1.0	<0.1	n.d.	n.d.	<0.01	n.d.	n.d.	0.010 ± 0.001
51 pumpkin	0.34 ± 0.01	1.4 ± 0.02	0.61 ± 0.01	n.d.	n.d.	0.049 ± 0.002	n.d.	n.d.	<0.01
52 linseed	0.42 ± 0.03	<1.0	<0.1	n.d.	0.056 ± 0.006	<0.01	n.d.	<0.01	<0.01
53 pumpkin	<0.3	1.0 ± 0.06	0.34 ± 0.02	n.d.	n.d.	0.027 ± 0.001	n.d.	n.d.	<0.01
54 pumpkin	<0.3	<1.0	0.27 ± 0.01	n.d.	n.d.	0.026 ± 0.001	n.d.	n.d.	<0.01
55 pumpkin	<0.3	1.1 ± 0.04	0.47 ± 0.03	n.d.	n.d.	0.033 ± 0.001	n.d.	n.d.	<0.01
56 pumpkin	<0.3	<1.0	0.27 ± 0.02	n.d.	n.d.	0.025 ± 0.001	n.d.	n.d.	<0.01
57 pumpkin	<0.3	<1.0	0.32 ± 0.02	n.d.	n.d.	0.037 ± 0.001	n.d.	<0.01	<0.01
58 pumpkin	0.66 ± 0.06	1.1 ± 0.07	0.49 ± 0.03	n.d.	<0.03	0.051 ± 0.002	n.d.	<0.01	<0.01
59 pumpkin	0.39 ± 0.04	1.0 ± 0.1	0.25 ± 0.02	n.d.	n.d.	0.022 ± 0.001	n.d.	n.d.	<0.01
60 pumpkin	0.39 ± 0.04	1.1 ± 0.08	0.27 ± 0.01	n.d.	n.d.	0.021 ± 0.001	n.d.	n.d.	<0.01
61 pumpkin	<0.3	1.2 ± 0.06	0.69 ± 0.05	n.d.	n.d.	0.044 ± 0.002	n.d.	<0.01	0.012 ± 0.001
62 pumpkin	n.d.	<1.0	n.d.	n.d.	n.d.	0.014 ± 0.001	<0.01	0.023 ± 0.001	n.d.
63 pumpkin	n.d.	1.1 ± 0.02	0.22 ± 0.01	n.d.	n.d.	0.030 ± 0.001	n.d.	0.011 ± 0.001	<0.01
64 pumpkin	n.d.	<1.0	0.16 ± 0.01	n.d.	n.d.	0.025 ± 0.001	n.d.	<0.01	<0.01
65 pumpkin	n.d.	<1.0	0.15 ± 0.01	n.d.	n.d.	0.017 ± 0.001	n.d.	<0.01	<0.01
66 salad oil	<0.3	<1.0	0.27 ± 0.03	n.d.	<0.03	0.034 ± 0.002	n.d.	<0.01	<0.01
67 hazelnut	<0.3	<1.0	0.28 ± 0.03	n.d.	0.070 ± 0.007	<0.01	n.d.	n.d.	0.11 ± 0.01
68 walnut	<0.3	<1.0	n.d.	n.d.	n.d.	<0.01	n.d.	n.d.	n.d.
69 pumpkin	n.d.	<1.0	0.16 ± 0.01	n.d.	0.065 ± 0.007	0.020 ± 0.002	n.d.	<0.01	<0.01

Table 4. Results of the comparative measurement of pumpkin seed oil with a SPME GC MS system based on a standard addition experiment.

Substance	Slope	Intercept	Conc. (mg kg^{-1})
2-Butanone	203469	44281	0.22
Acetone	120684	85312	0.71
Benzene	1127532	8083	0.007
m-/p-Xylene	7244505	91947	0.013
Pentane	6104	7615	1.3
Toluene	2117468	102874	0.049

the ground pumpkin seeds stood out with a high content in ethyl acetate (Figure 2(c)) as well as ethanol (Figure 3(b)). These could be degradation products of non-lipid plant material. In contrast to most of the substances which were not part of the initial scope of the method, the aldehydes hexanal (Figure 3(c)) and butanal (Figure 3(d)) increased quite uniformly in all oil samples. These compounds are common products of fat autoxidation which could explain this behaviour as well as the lack of any concentration increase in the pumpkin seeds. Other substances like (E)-oct-2-ene (Figure 3(a)), piperidine (Figure 3(e)) and propanol (Figure 3(f)) show significantly higher differences between the oils and might be more strongly linked to their composition.

Statistical evaluation

The occurrence of the volatile substances in the oil could largely be attributed to two groups of influencing factors: the composition of the oil, hence the fatty acid spectrum as well as other minor compounds and the production conditions which vary depending on the producer of the oil. Multivariate statistical analysis was used to test these hypotheses. The statistical software tool Mass Profiler Professional (Agilent Technologies Inc. and Strand Life Sciences Pvt. Ltd.) was used to evaluate the results of the thermal conditioning experiment and the sample measurements.

Fatty acid profile of the oil samples

The fatty acid profiles of the vegetable oils which had been used for the thermal conditioning experiment had been determined and are shown in Figure 4. The different oil types showed large variations in their fatty acid distribution which is reflected by the diverging analytical results regarding their content in volatile substances. Principal

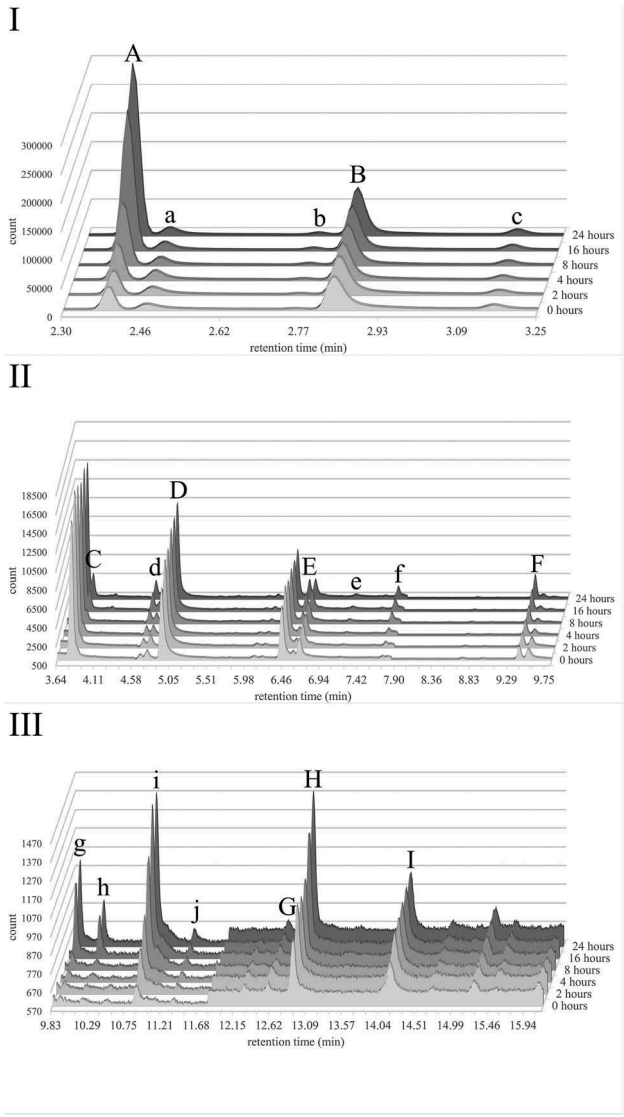

Figure 1. Comparative TIC's of pumpkin seed oil after different periods of thermal treatment at 120°C. Because of significantly varying signal intensities, the TIC was divided into three parts labelled with I (2.30–3.25 min), II (3.64–9.75) and III (9.83–15.94). Peaks labelled with uppercase letters show compounds which are part of the method's scope, whereas lowercase letters indicate peaks which respond to the SIM parameters in the method, but were not initially looked for. A NIST database search was performed for the identification of some of the signal (match factor >80%), however no analytical standard materials were available to confirm the results. Signals: (A) n-pentane; (B) acetone; (C) n-hexane; (D) 2-butanone; (E) n-heptane; (F) toluene; (G) ethylbenzene; (H) m- and p-xylene; (I) styrene. (a) ethanol; (b) propanol; (d) butanal; (g) (E)-oct-2-ene; (h) piperidine; (i) hexanal. Signals (c), (e), (f), (j) could not be reliably identified using the NIST 05 database.

component analysis (PCA) comparing the concentrations of both sample sets which had been measured with a gap of six months can be seen in Figure 5. A clear clustering of all the pumpkin seed oils and separation from all the other oil types can be observed. This clustering is independent of the producer of the oil and is therefore most likely due to the differences in the fatty acid spectrum and other minor ingrediencies of the oil. Unsaturated fatty acids are prone to degradation and form a variety of volatile alkanes like pentane and heptane (Choe and Min 2006). A PCA plot was also used to correlate the concentration of the three measured n-alkanes after 24 hours of thermal conditioning at 120°C with the relative content of unsaturated fatty acids. This is depicted in Figure 6. Pentane and other alkanes are typical thermal degradation products of unsaturated fatty acids and can therefore be expected to be found in vegetable oils which were subject to thermal pretreatment like roasting (Alencar et al. 1983; Schwab et al. 1988).

Differences between pumpkin seed oils due to the oil producer

The second critical influencing parameter besides the makeup of the oil is the production technique. Only the pumpkin seed oils were used for this evaluation to eliminate any deviations due to strong variations in the fatty acid spectrum. Every oil mill which produces pumpkin seed oil employs slightly different parameters for the roasting and pressing of the oil. These differences leave an imprint on the product. The concentrations of the following substances were used in a PCA to compare the various oil producers: acetone, benzene, toluene, ethylbenzene, m- and p-xylene, o-xylene, styrene, 2-butanone, ethyl acetate, pentane, hexane, and heptane. The samples of each sample set were produced within two months prior to the respective measurement and the two sample sets were measured six months apart. For the sake of clarity, the eight oil producers were split into groups of four and can be seen in Figure 7. Despite the two different sample sets, clustering of the pumpkin seed oils can be seen for some of the oil producers, especially producers #1, #5, #3 and #8. The samples of the other oil producers like #7 are more spread out which could be caused by variations in the production parameters. Figure 8 shows a comparison of the two sample sets in separate PCA plots. Due to the large variations even within one sample set, producer #7

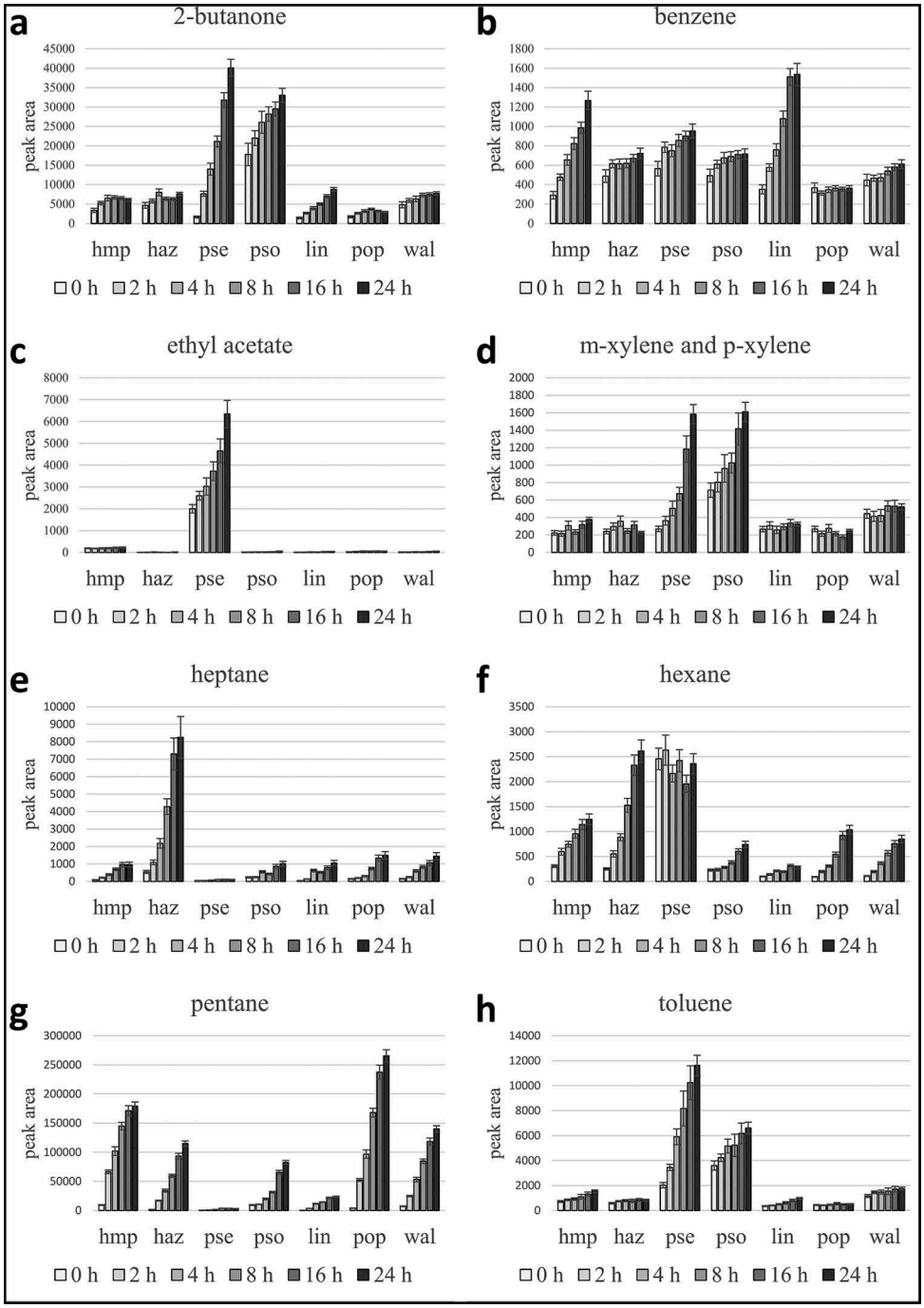

Figure 2. Change of the abundance of substances which were in the scope of the developed GC-MS in different oil types, over 24 hours of thermal treatment at 120°C. Abbreviations: **hmp** – hemp seed oil, **haz** – hazel nut oil, **pse** – ground pumpkin seeds, **pso** – pumpkin seed oil, **lin** – linseed oil, **pop** – poppy seed oil, **wal** – walnut oil. Results as mean values (RSD <20 %, n = 3).

was not included in this graph. The other producers, however, appear to achieve reproducible patterns of volatile substances in their pumpkin seed oils. Some producers like #6 and #8 seem to produce similar oils, which are clearly different from #1 and #3, as well as #2, #4 and #5. This is a clear indication of the influence of the production technique onto the spectrum of substances in the final product. These slight differences in the oil making process can be traced even over longer periods and leave a typical imprint in the oil. The focus of this project was the analysis of solvent residues, however subsequent projects could

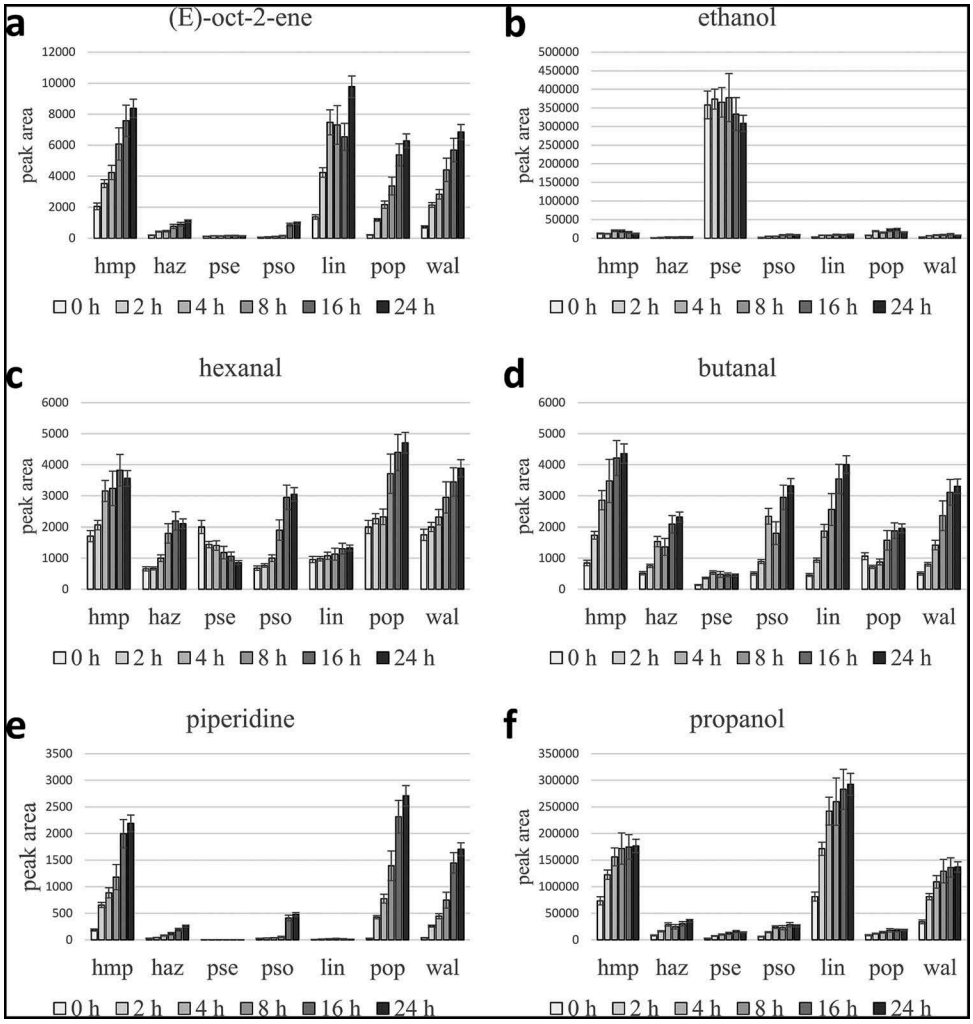

Figure 3. Change of the abundance of substances which were not in the scope of the method but which responded to the used SIM settings in different oil types, over 24 hours of thermal treatment at 120°C. Abbreviations: **hmp** – hemp seed oil, **haz** – hazel nut oil, **pse** – ground pumpkin seeds, **pso** – pumpkin seed oil, **lin** – linseed oil, **pop** – poppy seed oil, **wal** – walnut oil. Results as mean values (RSD <20 %, n = 3).

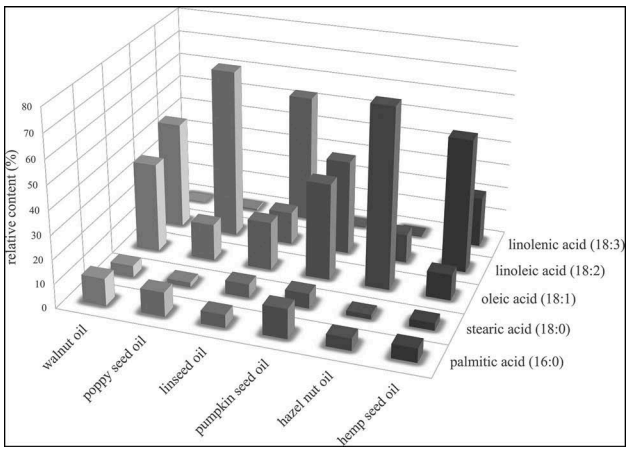

Figure 4. Fatty acid profile of the oils which were subjected to thermal treatment at 120°C (external analysis, only most abundant fatty acids displayed).

expand the scope of the method as well as monitor various production parameters. Obtained results could then be used to specify the influence of the different production steps.

Summary and conclusion

Multiple Styrian oil producers were accused of introducing solvent residues in their products and that they might have used solvent extraction for producing their oils. A project was initiated to investigate the accusations. A HS-GC-MS method which was developed and validated for the project was used to measure 69 unrefined oil samples which were split into two sample sets that were measured with a gap of six months. Most of the

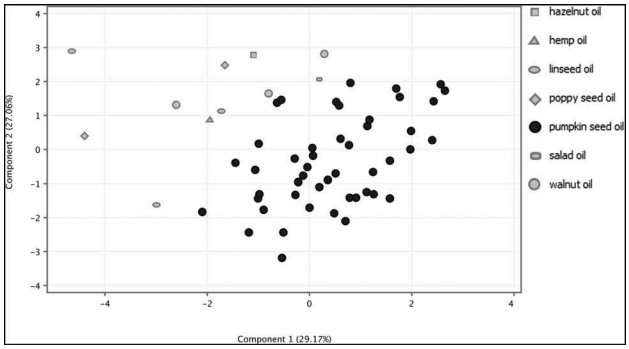

Figure 5. PCA comparing the different oil types regarding the results of the measurements of both sample sets. Pumpkin seed oils are depicted with darker symbols.

oils m-xylene and p-xylene were found as well. All the findings were in the in the µg kg^{-1} to low mg kg^{-1} range. Complementary measurements of a pumpkin seed oil which were performed at the Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, using SPME-GC-MS confirmed the findings. The detected substances and their concentrations showed patterns regarding the oil type. This led to the assumption that the substances form intrinsically during the production process. A thermal conditioning experiment which

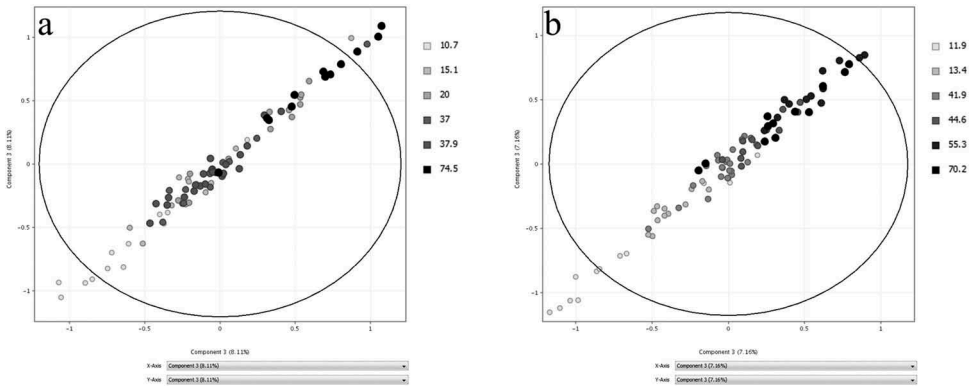

Figure 6. PCA plots comparing the relative contents of oleic acid (a) and linoleic acid (b) in vegetable oils with the concentration of n-pentane, n-hexane and n-heptane after 24 hours of conditioning at 120°C.

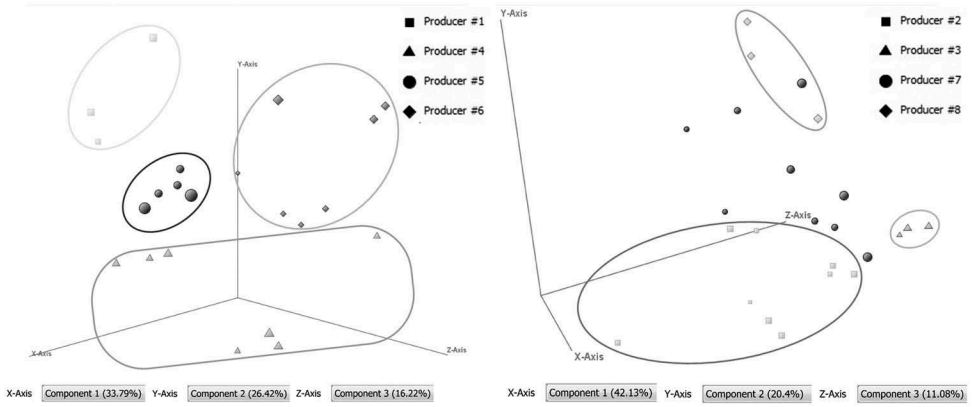

Figure 7. PCA plots of the relation between the spectrum of volatile substances in the pumpkin seed oil samples and the producer of the oil. Both sample sets were evaluated and the producers were split into two groups of four.

samples were pumpkin seed oil, however oils from other oil seeds were analysed as well. A variety of different volatile compounds which are commonly used as solvents were found in the oils. The most abundant analytes were 2-butanone, acetone, pentane and toluene. In most pumpkin seed exposed different oil types to a temperature of 120°C over 24 hours, showed that the concentration of most of the analytes increased over time. The temperature of 120°C was selected since pumpkin seeds are roasted at this temperature, however the exact temperature and the roasting

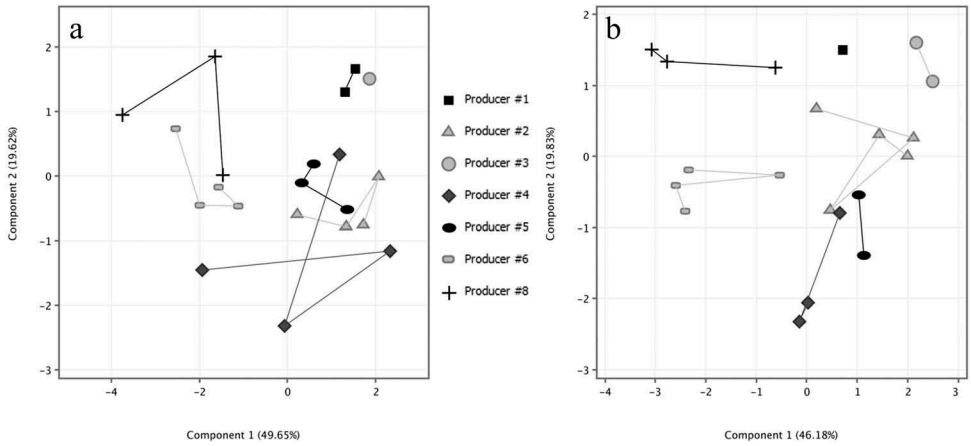

Figure 8. PCA plots of the relation between the spectrum of volatile substances in the pumpkin seed oil samples and the producers of the oils for the two individual sample sets. Sample set 1 is shown in graph A and sample set 2 is shown in graph B. Producer #7 was excluded from the evaluation.

time varies from one producer to another. Multivariate data analysis of the results of all the samples showed that pumpkin seed oils had a characteristic pattern of volatile substances which are different from all the other oils. It also showed the correlation of the concentration of n-alkanes in an unrefined oil and the degree of unsaturation of the fatty acids. Many of the analytes could therefore be traced back to triglyceride degradation due to autoxidation or harsh conditions during the oil production. Principal component analysis showed that these conditions leave a distinct imprint on the samples since it was possible to cluster the oil producers in both sample sets. This showed that traditionally produced and unrefined vegetable oils have a certain content of volatile substances which can be misinterpreted as solvent residues. These are technically unavoidable and form alongside many typical flavouring substances. Legislative organisations should consider specifying the rules to incorporate the occurrence of the compounds and to eliminate uncertainty for the oil producers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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