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Determination of Aldehydes and Ketones in Oily Matrices using a Novel Dynamic Headspace Sampler Coupled to GC/MS

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Introduction

Fatty acids are of key importance to the food industry. Especially long chain polyunsaturated fatty acids (LC PUFA) are receiving more and more attention due to their positive influence on human health. LC PUFA refined from natural oils are frequently added to food products to gain a positive health effect. Since LC PUFA are rather unstable and prone to oxidation, the quality of oils and fats has to be controlled. Certain aldehydes, ketones and other compounds are markers for oil and fat quality (Figure 1). Some of these have unpleasant odors and/or tastes (e.g. fishy) which are not acceptable to consumers. The compound 4-heptenal, for example, adds a detectable fishy odor and taste to food products at concentrations as low as 10 ng/g. This and other marker compounds therefore have to be monitored in the ng/g range by the food industry.

Static Headspace GC/MS analysis is mostly not sensitive enough to determine the markers for oil and fat quality at the levels required. Gas phase SPME provides better sensitivity and is widely used for monitoring purposes.

In this paper we report on the use of the dynamic headspace technique for the analysis. A novel dynamic headspace system (DHS), was employed to determine several aldehydes, ketones and other fatty acid degradation markers in oily samples.

Samples were kept in standard 20 mL screw cap vials. During dynamic headspace sampling inert gas (nitrogen) was purged through the headspace of the vial. Analytes were purged from vial and concentrated on adsorbent packed tubes placed in the gas exit. Adsorbent tubes can be filled individually and are exchangeable, making it possible to use a new tube for every sample. After sampling a defined volume, the tubes were transferred to a thermal desorption system where they were desorbed, transferring the analytes to the GC/MS system. The entire process was automated using the GERSTEL LabWorks Platform (Figure 2).
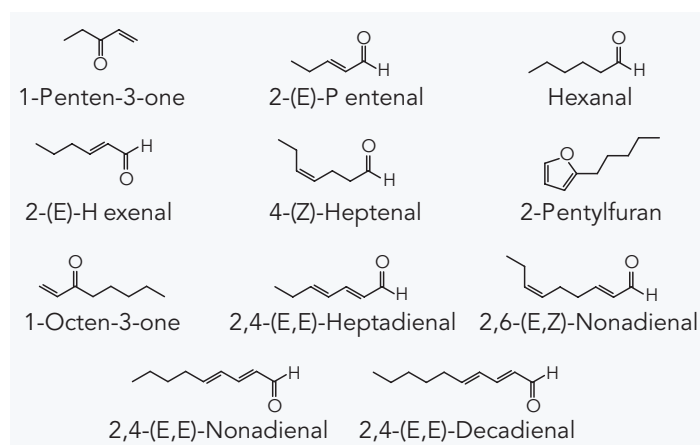

Figure 1: Typical fatty acid degradation compounds.

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Figure 2: GERSTEL LabWorks Platform with DHS option mounted on an Agilent Technologies 7890 GC / 5975 MSD.

Figure 3: Closeup view of the GERSTEL Dynamic Headspace (DHS) module.

Experimental

Oil samples in the amount of 1 g each were weighed into standard 20 ml screw cap vials. This was the only sample preparation needed for the analysis. All other steps were performed by the autosampler, which was conveniently operated through the GER-STEL MAESTRO software.

Due to the difficult matrix, standard addition calibration had to be used for analyte quantification. A stock solution of 1 µg/µL in hexane was prepared. Spiking solutions were prepared by diluting the stock solution with appropriate volumes of hexane resulting in concentrations from 5 to 500 ng/µL for calibration. A 1 µL aliquot of the appropriate calibration solution was added to each 1 g sample by the autosampler. After an equilibration period at room temperature the vial was transported to the GERSTEL DHS module and equilibrated in the shaker at 70 °C for 4 minutes. DHS extraction was then performed using a gas flow of 50 mL/min for 10 minutes, concentrating analytes on a Tenax-filled adsorbent tube. Following the extraction, the tube was immediately desorbed in the GERSTEL Thermal Desorption Unit (TDU). A GERSTEL Cooled Injection System (CIS) was used to refocus analytes at low temperature and to transfer them onto the GC separation column.

Analysis Conditions
LabWorks Platform

Trap	Tenax
DHS	30 °C trap temperature
	70 °C incubation temperature
	500 mL purge volume
	50 mL/min purge flow
TDU	splitless
	40 °C; 720 °C/min to 280 °C (5 min)
PTV	0.01 min solvent vent (70 mL/min)
	split 2.5:1
	-150 °C; 12 °C/s to 270 °C (7 min)

Agilent 7890 GC

Column	30 m DB-624 (Agilent)
	d_i = 0.25 mm d_f = 1.4 µm
Pneumatics	He, constant flow, 1.5 mL/min
Oven	40 °C (1 min); 4 °C/min to
	170 °C; 30 °C/min to 240 °C (5 min)

Agilent 5975 MSD

mode	SIM

Results and discussion

A total of ten different samples were analyzed using the described method, most of them vegetable oils. Additionally two fish oils and one milk powder for babies enriched with LC PUFA were analyzed. The concentrations of most analytes were in the range of 1-100 ng/g as can be seen in table 1.

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Table 1: Analytical results for the analyzed samples.

Analyte	RT [min]	m/z	Olive oil 1 fresh [ng/g]	Olive oil 2 old [ng/g]	Olive oil 3 old [ng/g]	Rapeseed oil 1 fresh [ng/g]	Rapeseed oil 1 fresh [ng/g]	Rapeseed oil 2 old [ng/g]	Corn oil old [ng/g]	Fish oil capsules, fresh [ng/g]	Fish oil old [ng/g]	Milk powder fresh [ng/g]
1-Penten-3-one	7.530	55	104.5	27.6	43.9	17.5	1.1	5.0	1.4	19.8	136.3	1.6
2-(E)-Pentenal	10.432	83	45.9	60.0	232.2	13.6	1.5	15.7	3.5	22.5	203.7	0.1
Hexanal	11.892	56	>500	>500	>500	>500	26.1	244.7	>500	29.5	611.6	84.4
2-(E)-Hexenal	14.532	83	>500	133.2	>500	17.9	0.4	19.9	6.3	54.1	78.5	0.8
4-(Z)-Heptenal	16.010	94	0.3	na	na	na	0.2	4.9	1.3	5.6	55.6	nd
2-Pentylfuran	18.858	81	0.1	185.1	105.8	35.6	0.5	0.3	31.8	11.5	4.0	0.6
1-Octen-3-one	19.304	70	1.3	51.4	38.6	16.8	nd	2.3	4.1	3.4	3.8	2.8
2,4-(E,E)-Heptadienal	21.484	81	29.0	65.8	577.5	14.7	12.0	90.0	3.8	61.2	190.1	1.3
2,6-(E,Z)-Nonadienal	26.900	70	nd	1.7	16.2	4.3	nd	nd	nd	33.2	62.6	8.6
2,4-(E,E)-Nonadienal	29.364	81	2.9	42.8	111.1	63.7	0.9	6.9	2.5	4.9	19.8	5.9
2,4-(E,E)-Decadienal	32.964	81	47.3	378.3	647.3	9.2	5.9	52.6	15.0	8.6	6.0	0.2

nd=not detected, na=not applicable due to coelution with an other compound

The results show that different brands of fresh rapeseed oil (brand 1, brand 2) have very different levels of fatty acid degradation markers. This means that the quality of an oil can be assessed using the DHS technique. During aging of oils the concentrations of certain marker compounds increase. The resulting pattern varies from oil to oil. Examples of the effects of aging can be seen in figures 4 and 5 where a fresh and an old rapeseed oil of the same brand and a fresh and an old fish oil are compared respectively.
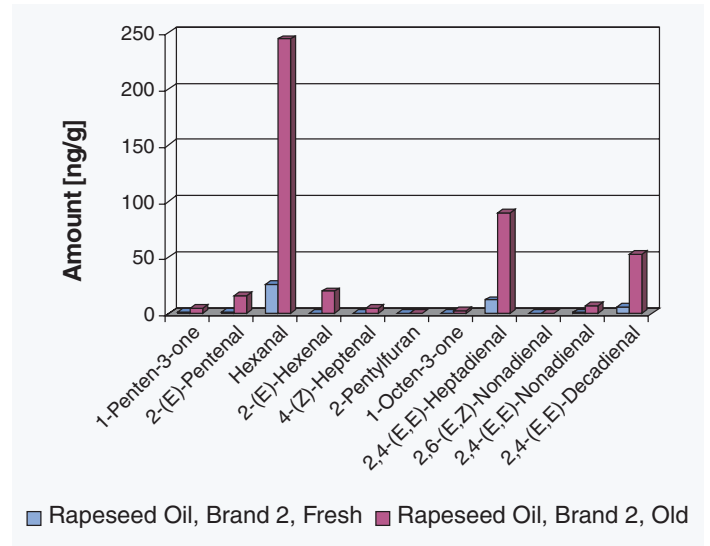

Figure 4: Comparison of concentrations of analytes in fresh and old rapeseed oil (same brand).
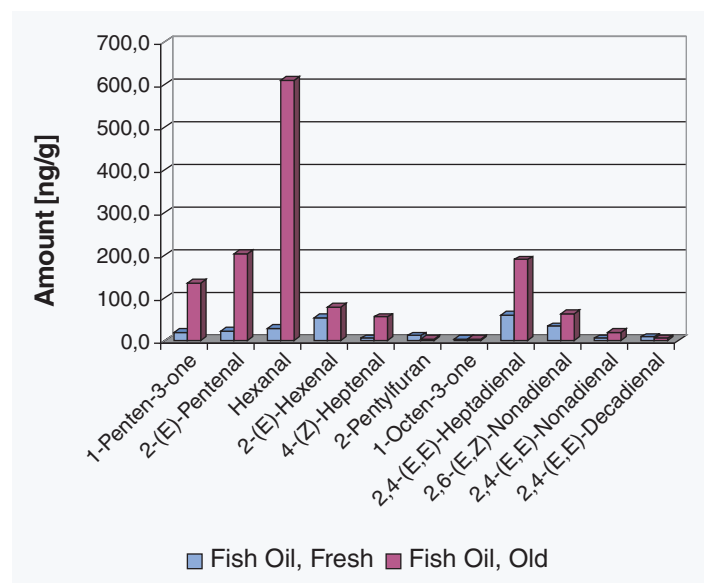

Figure 5: Comparison of concentrations of analytes in fresh and old fish oil.

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Standard addition calibration curves for various compounds in an oil sample are shown in figures 6 and 7. It can be seen that these are linear up to 500 ng/g added standard, of course depending on the original concentration levels in the sample. For most compounds, correlation coefficients were around 0.999 which is very good. Hexanal (concentration level higher than 500 ng/g) and 4-(Z)-heptenal (coelution) are not included in these sets of calibration curves.
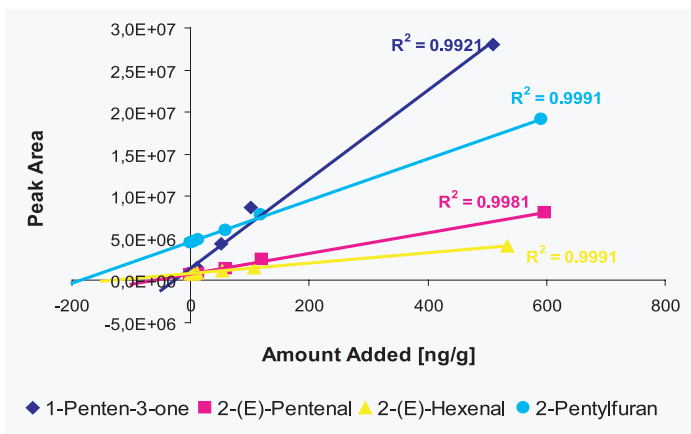
Figure 6: Calibration curves for analytes 1-4 (table 1) in olive oil, brand 2, old.
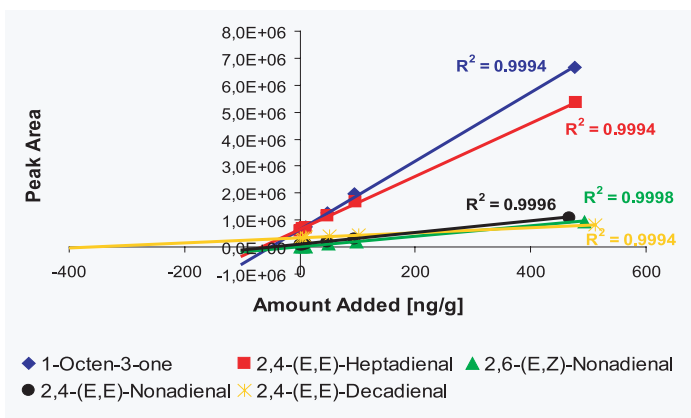
Figure 7: Calibration curves for analytes 5-9 (table 1) in olive oil, brand 2, old.

Figure 8 shows overlays of standard addition calibration chromatograms for 2,4-(E,E)-heptadienal in an olive oil sample (brand 2, old).
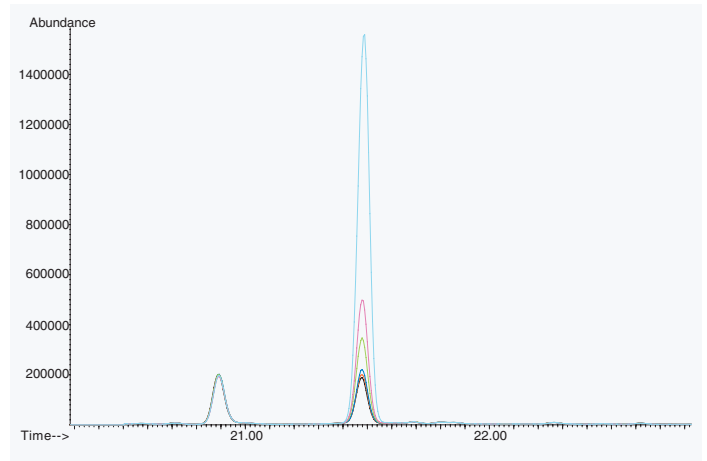
Figure 8: Overlay of standard addition chromatograms (0-500 ng/g) of ion trace m/z=81 for 2,4-(E,E)-heptadienal in an olive oil sample (brand 2, old).

The repeatability of the analysis was tested using a rapeseed oil (brand 2, fresh) containing relatively low analyte amounts, which was spiked with 5 ng/g of the analytes. The relative standard deviations (RSDs) resulting from five replicates are shown in figure 9. Even at this very low concentration, RSDs for most compounds were well below 5%. Please note, that the results for the compounds listed with higher RSDs are in a very respectable range when compared with other published work [1].
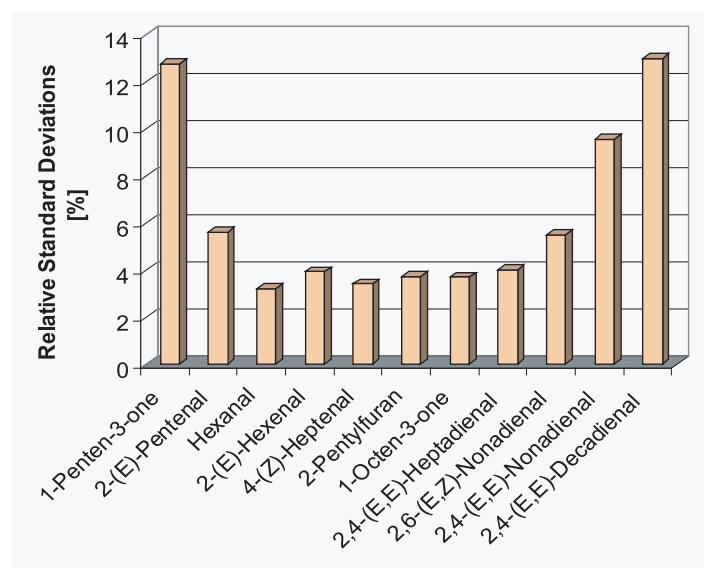
Figure 9: Relative standard deviations of five repeated measurements of rapeseed oil (1g, brand 2, fresh) spiked with 5 ng/g of analytes.

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In order to test the ruggedness of the method, a second calibration, using only half the original sample weight, was performed. The analytical results of the two calibrations match quite well as can be seen in figure 10.
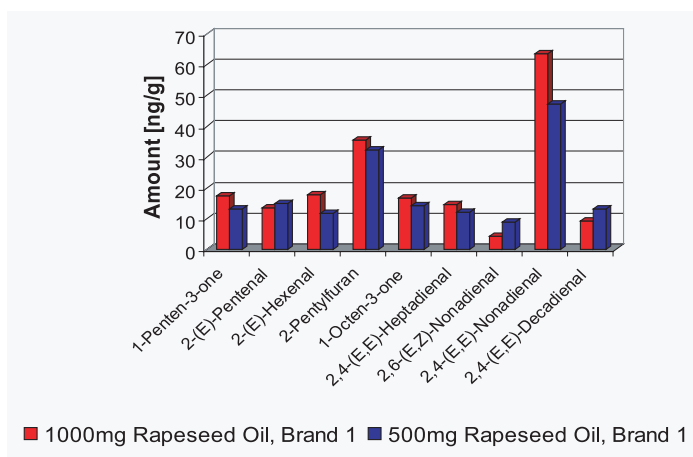

Figure 10: Analysis results for 1000 and 500 mg sample weight.

As an example, the calibration curves for 2,4-(E,E)-heptadienal for the two sample weights (1000 and 500 mg) are shown in figure 11.
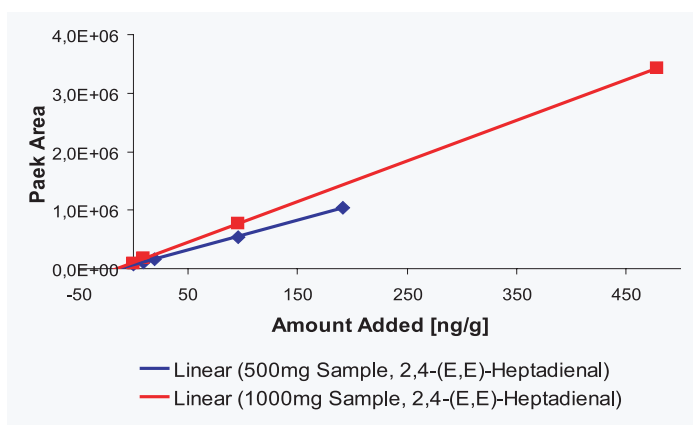

Figure 11: Standard addition calibration curves for 2,4-(E,E)-heptadienal for 1000 and 500 mg sample weight.

A blank determination using an empty vial following a highly concentrated sample showed that the method has relatively low carryover. The blank chromatogram was compared to the most concentrated calibration sample which was run just prior to the blank (figure 12). For most compounds carryover was around 0.01%.
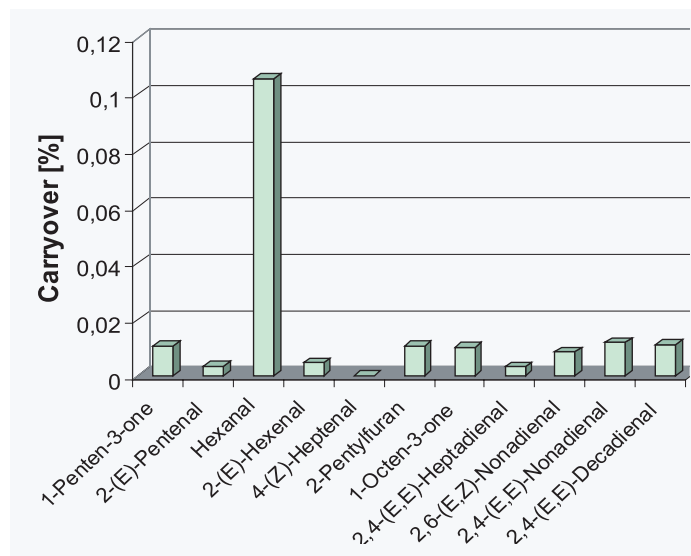

Figure 12: Carryover seen in a blank run following directly after a 500 ng/g calibration sample.

Limits of determination for the method could be estimated from the blank chromatogram. These are in the range between 0.05 and 0.5 ng/g

Conclusions

A method for the determination of select aldehydes and ketones as markers for fatty acid degradation has been developed. The method has the following key features:

■ Dynamic headspace (DHS) extraction with automated standard addition calibration

■ The analysis is fully automated, weighing of the sample is the only manual step

■ Good correlation for calibration curves ($r^2=0.999$ in many cases and RSDs mostly < 5%)

■ Limits of determination estimated between 0.05 and 0.5 ng/g

■ Can be applied to other aldehydes, ketones etc.

■ Valuable tool for assessing the quality of oils, fats and products containing fatty acids

References

[1] Bao et al., Analyst, **1999**, 124, 459–466