Determining markers for fatty acid decomposition

Fighting rancidity

Assessing the quality of food oils, fats and products that contain fatty acids may be cumbersome, but it is necessary in order to safeguard product quality and consumer safety. Scientists from GERSTEL have developed a new and more efficient method for monitoring quality markers in oily matrices: Oxidation products such as aldehydes and ketones are determined using automated Dynamic Headspace (DHS) coupled with GC/MS.

Text: Guido Deußing

Salmon, bluefish, trout, walnuts, rapeseed oil, sesame seeds, sunflower seeds, soybeans, corn, vegetable oil-based spread... this is not a shopping list, but these items are recommended as part of a healthy and nutritious diet. The reason for such a recommendation is that these foods contain long-chain polyunsaturated fatty acids (LCPs). LCPs can be divided into two categories: n-6-fatty acids (formerly known as omega-6-fatty acids) among these linoleic acid and its derivatives, and n-3-fatty acids (formerly known as omega-3-fatty acids) to which group the α-linolenic acid and its derivatives belong.

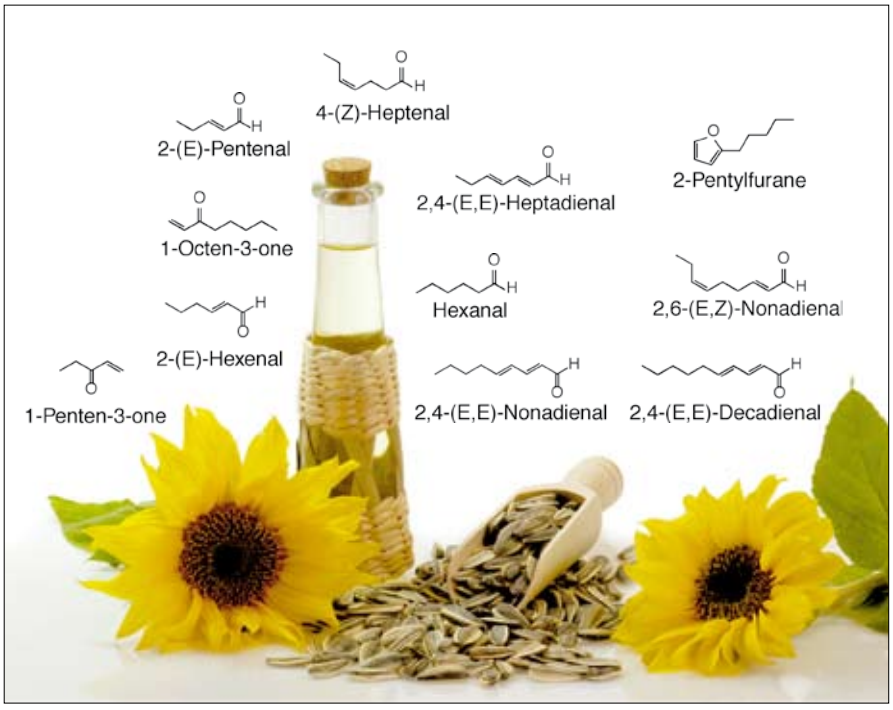
LCPs are said to have many beneficial properties. Provided to us via our mother's milk, they provide an essential contribution to the development of the brain, nervous system and vision in the child. At any age, LCPs are known to benefit our heart and circulatory system and they prevent or reduce arteriosclerosis and related illnesses. A deficiency of essential fatty acids can have dire negative consequences starting with skin conditions, such as calloused skin, increased susceptibility for infections, reduced growth, hair loss, and reduced blood plate-count.

It is hardly surprising that LCPs are extracted from natural products and added to foods in order to provide health benefits via pre-natal and post-natal baby nutrition. These extracts are also used to enrich foods that are low in LCPs, such as standard bread products, and turn these into more valuable so-called functional foods that have health benefits.

Strength and Weakness

LCPs strengthen us, but are themselves of weak constitution. Getting too much "fresh air" doesn't help them either. Oxygen molecules attack and destroy their double bonds and degradation products are formed. And this doesn't exactly go unnoticed, since compounds with unpleasant odors and very low odor thresholds are formed. Degradation products such as aldehydes and ketones, 4-heptenal for one, are among the oxidative degradation products formed from the fatty acids. A concentration of less than 10 ng per gram is typically sufficient to give an oil or food a distinct rancid smell or flavor.

For most foods, the contact with oxygen cannot be prevented, only reduced, but degradation reactions can at least be slowed by storing foods at low temperatures. In the end, freshness of food oils and fats should be monitored. How then to monitor these reactions, when odor thresholds are so very low? Instrumental techniques are required that can extract and concentrate analytes without accelerating the very process they are meant to monitor, i.e. without heating the sample too much in the process. According to Oliver Lerch, Ph.D., application scientist at GERSTEL, Static Headspace (HS) coupled with GC/MS is a useful technique for the determination of volatile compounds. However, for the determination of oxidation products in oily foods, detection limits reached using Headspace GC/MS are much too high; it is simply not possible to monitor these compounds at the required concentration levels using Static Headspace. More sensitive techniques are needed, such as Headspace Solid Phase Micro-Extraction (HS-SPME), which relies on a fiber coated with sorbent to concentrate analytes from the sample headspace or the Dynamic Headspace (DHS) technique, which purges and concentrates analytes onto an adsorbent trap. "Of these more sensitive techniques, we achieved the best results", says Lerch, "when we used the Dynamic Headspace (DHS) technique for the determination of oxidation markers such as aldehydes and ketones".



Structure of the determined fatty acid decomposition products

Theory guides, experiment decides

Based on a customer request, Dr. Lerch and his colleagues in the Analytical Services Department of GERSTEL investigated ten different oil samples using automated DHS coupled with GC/MS. Most of the samples were vegetable oils, among them olive oils and rapeseed oils from different producers. Two of the samples were fish oils.

The GERSTEL scientists mainly focused on eleven compounds that are known degradation products of LCPs: 1-pentene-3-one, 2-(E)-pentenal, hexanal, 2-(E)-hexenal, 4-(Z)-heptenal, 2-pentylfurane, 1-octene-3-one, 2,4-(E,E)-heptadienal, 2,6-(E,Z)-nonadienal, 2,4-(E,E)-nonadienal and 2,4-(E,E)-decadienal. One gram of each oil sample was stored in a 20 mL screw cap headspace vial. "Weighing in the samples was the only manual preparation step we had to perform", says Oliver Lerch, "and if we had used the automated weighing option for the MPS, even this step could have been automated" (cf. GERSTEL Solutions Worldwide No. 8). All further sample preparation steps, including adding an internal standard, performing Dynamic Headspace extraction, and introducing the concentrated analytes to the GC/MS were performed automatically.

Some details: Standards containing from 5 to 500 ng/µL of the target compounds were prepared from a 1 µg/µL stock solution by dilution with hexane. 1 µL of standard solution was added to each of the vials containing 1 g of oil sample. The vials were placed in the MPS sample trays and successively transferred into the DHS station agitator, where they were kept at 70 °C for 4 minutes for equilibration. DHS extraction was then performed for 10 minutes using a 50 mL/min flow of nitrogen to purge and transfer analytes onto a replaceable Tenax TA adsorbent trap for concentration.

Oliver Lerch, Ph.D.,
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The user can specify whether a new trap is used for each sample and of course which adsorbent is used, the tubes are available with any standard adsorbent. Following dynamic headspace extraction and analyte concentration, the adsorbent tube is transferred to the GERSTEL Thermal Desorption Unit (TDU). Analytes are desorbed in the TDU, transferred to the Cooled Injection System (CIS), where they are again focused, and finally transferred in a narrow band to the GC/MS system for identification and quantification.

Fatty acid decomposition provides a clear picture of product quality

Many samples were analyzed using the GERSTEL DHS in order to prove its usefulness over the entire concentration range of decomposition products (generally from 1 to 100 ng/g) that represents fresh, aged or slightly rancid product.

As an example, Lerch mentions rapeseed oil products, which were analyzed as described above and categorized: Fresh oils mostly had a very low concentration of decomposition products. When such oil had been stored under normal household conditions for six months, however, levels had increased significantly (cf. table 1). Apart from the aspect of aging, large differences in freshly purchased oils from different producers were demonstrated (cf. table 2). "The statistics supported our findings nicely", says Lerch, "the standard addition calibration curves ranged up to 500 ng/g with excellent linearity; most correlation coefficients were at 0.999". Standard deviation

Analyte	RT [min]	m/z	rapseed oil No.1 (fresh) in ng/g	rapseed oil No.1 (aged) in ng/g
1-Pentene-3-one	7.530	55	1.1	5
2-(E)-Pentenal	10.432	83	1.5	15.7
Hexanal	11.892	56	26.1	244.7
2-(E)-Hexenal	14.532	83	0.4	19.9
4-(Z)-Heptenal	16.010	94	0.2	4.9
2-Pentylfurane	18.858	81	0.5	0.3
1-Octen-3-one	19.304	70	nd	2.3
2,4-(E,E)-Heptadienal	21.484	81	12	90
2,6-(E,Z)-Nonadienal	26.900	70	nd	nd
2,4-(E,E)-Nonadienal	29.364	81	0.9	6.9
2,4-(E,E)-Decadienal	32.964	81	5.9	52.6

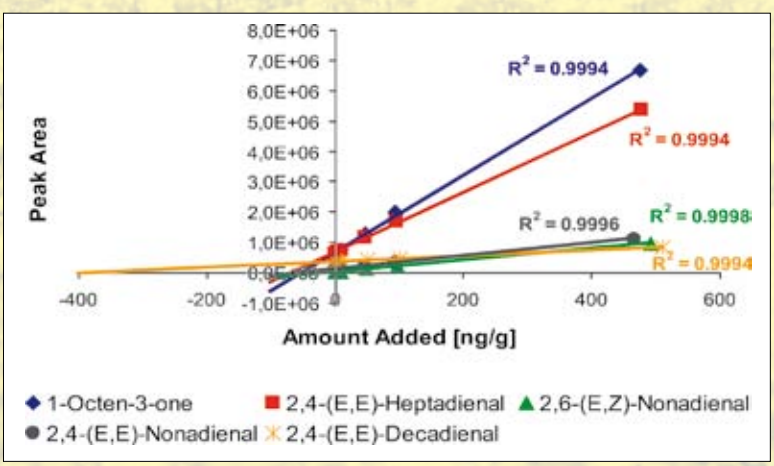
nd = not detected

Table 1: Comparison of fresh and aged rapeseed oil, which had been stored for 6 months under normal household conditions.

Analyte	RT [min]	m/z	rapseed oil No.1 (fresh) in ng/g	rapseed oil No.2 (fresh) in ng/g
1-Penten-3-one	7.530	55	1.1	17.5
2-(E)-Pentenal	10.432	83	1.5	13.0
Hexanal	11.892	56	26.1	> 500
2-(E)-Hexenal	14.532	83	0.4	17.9
4-(Z)-Heptenal	16.010	94	0.2	nd
2-Pentylfurane	18.858	81	0.5	35.6
1-Octen-3-one	19.304	70	nd	16.8
2,4-(E,E)-Heptadienal	21.484	81	12	14.7
2,6-(E,Z)-Nonadienal	26.900	70	nd	4.3
2,4-(E,E)-Nonadienal	29.364	81	0.9	63.7
2,4-(E,E)-Decadienal	32.964	81	5.9	9.2

nd = not detected

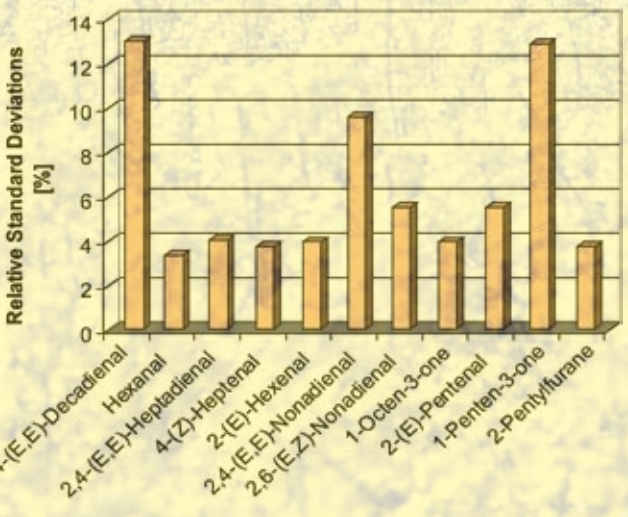
Table 2: Comparison of two fresh rapeseed oils from different producers.



The standard addition calibration curves for the different compounds in an oil sample are linear up to 500 ng/g. The correlation coefficients were around 0.999 for almost all compounds.

Analytical conditions

Adsorbent:	Tenax TA
DHS:	Trap temperature: 30 °C; Incubation temperature: 70 °C; extraction/purge volume: 500 mL N_2
TDU:	Splitless mode; Temperature program: 40 °C (0 min) – 720 °C/min to 280 °C (5 min)
CIS:	TDU desorption flow: 70 mL/min Analyte transfer: Split 2.5:1 Temperature program: -150 °C (0 min); 12 °C/s to 270 °C (7 min).
Column:	DB-624 (Agilent Technologies); Length: 30 m; I.D. = 0.25 mm; df = 1.4 µm
Carrier gas:	He, constant flow: 1.5 mL/min
GC oven program:	40 °C (1 min); 4 °C/min to 170 °C; 30 °C/min to 240 °C (5 min)
MSD mode:	Selected Ion Monitoring (SIM)



Repeatability was tested with fresh rapeseed oil which had been spiked with 5 ng/g of each analyte. The RSD for five runs was under 6 % for most compounds, this was at least as good as the results obtained when performing the analysis with HS-SPME.

For the determination of quality markers from fatty acid decomposition, the GERSTEL scientists relied on an automated DHS-GC/MS system. The instrument set-up has been implemented successfully for this application in customer laboratories.

and repeatability was tested with fresh rapeseed oil that had been spiked with 5 ng/g of each target analyte. The RSD for five runs was under 6 % for most compounds. The method proved to be robust and run-to-run carry-over was below 0.01% for almost all compounds.

When using the DHS-GC/MS technique, the analyst is able to get a clear and unequivocal picture of LCP decomposition and thereby of the quality and freshness of oils, fats, and foods that contain fat. The concentration of aldehydes and ketones that are "marker compounds" for fat decomposition will rise over time as the product ages and this makes it easy to determine product freshness. The limits of determination for the marker compounds are in the range from 0.05 to 5 ng/g. The excellent correlation is also significant, "it is proof that the method is well suited for quality control of foods", says Oliver Lerch.