



Solid-Phase Microextraction (SPME) in the Fish Oil Industry

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A review of the role of SPME in quality control (QC) in the fish oil industry.

This article provides an overview of the use of solid-phase microextraction (SPME) in the fish oil industry to monitor oxidation products in marine oils. SPME was developed by Janusz Pawliszyn et al.¹ in the 1990s and its applications in the field of pharmaceutical analysis, food analysis and environmental analysis have been extensively reviewed.² By combining sampling and sample preparation into one step, SPME has been shown to provide many benefits, including speed, simplicity of operation, ease of automation, as well providing a solvent-free sample preparation method.

The fish oil industry is developing food supplements and functional food products that contain omega-3 fatty acids. The use of SPME to measure the quality of fish oil will be assessed in this review.

Oxidation of Fish Oil

Omega-3 fatty acids are polyunsaturated fatty acids that have the final double bond in the hydrocarbon chain between the third and fourth carbon atoms from the methyl end of the molecule. Examples of such fatty acids include (5Z,8Z,11Z,14Z,17Z)-eicosa-5,8,11,14,17-pentaenoic acid (EPA) and (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-

hexaenoic acid (DHA). These fatty acids are in demand, because of the increasing amount of scientific data illustrating the positive health contribution of omega-3 fatty acids on cardiovascular disease risk factors,³ inflammation and inflammatory diseases,⁴ and cognition.⁵

Omega-3 fatty acids can be attached to triglycerides found in fish oil, but they can also be attached to phospholipids, which are abundant in krill oil. Phospholipids with omega-3 fatty acids attached are of particular interest because their bioavailability is better than regular fish oil.⁶ Omega-3 fatty acids are highly susceptible to oxidation, especially in the presence of air and light. Oxidation takes place through several stages and initially involves the formation of free radicals caused by metals, light and/or peroxides. The propagation phase follows, where free radicals react with oxygen, resulting in the formation of hydroperoxides and more radicals until the termination phase where non-radical products are formed.

The hydroperoxides are called primary oxidation products and are unstable. They decompose eventually into secondary oxidation products, which are volatile and have a strong and unpleasant smell and taste, characteristic of

rancid marine oils.⁷ The smell and taste of rancid fish affects the consumption of marine oil food supplements, which is something the industry obviously wants to avoid. The problem of rancidity can be solved by presenting the oil in a capsule or by adding a masking agent, but for functional food products only non-oxidized marine oils are acceptable.

Determining Fish Oil Quality

The industry is developing new methods of refining oils that have superior quality and are testing the smell/taste profile using trained and qualified taste panels. This is an expensive and time-consuming process and the need for a laboratory method to identify and quantify molecules that taste panel members can detect is required. Classical lipid chemistry methods to measure oxidation have been investigated, however, it has been found that peroxide value,⁸ anisidine value, 2-thiobarbituric acid value and conjugated dienes do not correlate the oxidation status with the sensory perception of a taste panel.⁹ Lipid peroxides form a range of breakdown products that result in a highly complex headspace. It comprises of unsaturated and saturated compounds such as aldehydes, ketones, alcohols and hydrocarbons.¹⁰ Some of the compounds have no flavour, others have a distinct smell and taste of, for example, rancid fish, metal, paint, cucumber or plastic. The odour threshold value ranges from 90–2000 µg/g for hydrocarbons to 0.002–7.000 ng/g for unsaturated ketones.¹¹ Macfarlane et al. identified three key components that were responsible for the fishy smell and taste, namely 2,6-nonadienal, 4-heptenal and 3,6-nonadienal. Based on these observations a model for the fishy taste was developed that was called the fatty acid smell and taste (FAST) index. Jacobsen et al. claim that the FAST index is insufficient to describe the fishy flavour¹² because it does not consider sensory descriptors such as metallic, rancid and "paint-like". It has been shown that at least 60 volatiles are present in fish oil-enriched emulsion and that the most potent odorants were 1-penten-3-one, (Z)-4-heptenal, 1-octen-3-one, 1,5-octadiene-3-one, (E,E)-2,4-heptadienal and (E,Z)-2,6-nonadienal.¹³ Using partial least-squares regression and multiple linear regression, Jacobsen et al. found the importance of (E,Z)-2,6-nonadienal and 1-pentene-3-one for causing off-flavours. Furthermore, they also suggested that the compounds could be useful markers for fishy and metallic off-flavours in fish and fish oil-enriched food products. As the volatiles responsible for causing off-flavours have been identified, there is a potential for an instrumental method that can replace the human taste

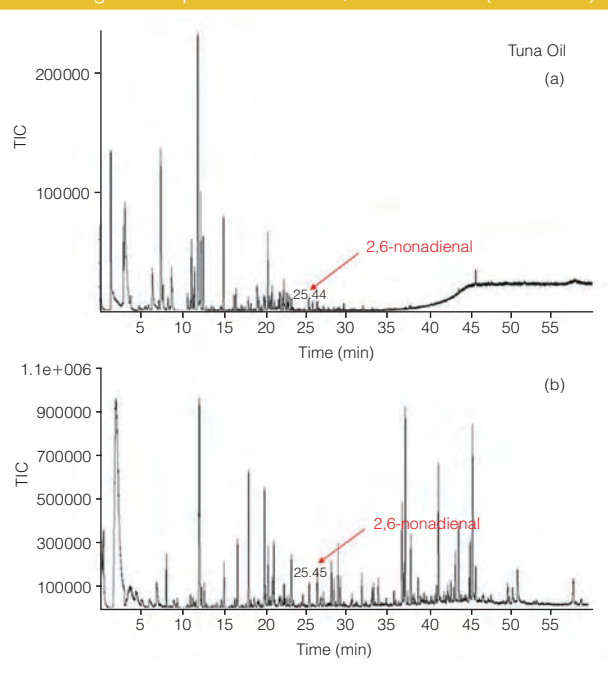
panel. Measurements of volatiles present in olive oils for quality control (QC) purposes have been performed for a number of years.¹⁴ Cavalli et al.¹⁵ compared static headspace, headspace SPME and headspace sorptive extraction and direct thermal desorption techniques to determine the chemical composition of different olive oils. They concluded that for the quality control of olive oil, SPME is the preferred method because of its operational simplicity, repeatability and low cost. Headspace SPME is more selective than direct SPME and is easier to automate, faster than dynamic headspace and has better precision and linear response.²⁰

To determine the sensory profile of olive oil, electronic sensing techniques based on gas sensors have been tested and shown to correlate very well with a human taste panel.¹⁶ Unfortunately, this is not the case for marine oils according to the observations performed by others.⁹ The reason may be that in fish oil there are volatiles present with very low flavour threshold levels that are not detected by an electronic nose. Detection limits (DLs) in the low ng/g range are required for compounds such as (E,Z)-2,6-nonadienal, (Z)-4-heptenal and 3,6-nonadienal.

SPME to Determine the Quality of Fish Oil

Previously, it was shown that headspace SPME could be used in the quality control of oxidized fish oil¹⁷ and

Figure 1: Profile of volatiles obtained from tuna oil using either headspace SPME (a) or headspace stir bar sorptive extraction (b). In both examples the temperature programme used was: 40 °C (hold 2 min), then at 5 °C/min to 250 °C (hold 16 min). SPME fibre = PDMS/DVB/Carboxen, 50/30 µm fibre. Extraction conditions = 45 min at 40 °C, desorption 270 °C (3 minutes). Incubation temperature = 45 °C. Incubation time = 15 min. Agitation = 250 rpm. Thermal desorption 270 °C (3 min). For the headspace sorptive extraction a 10 mm PDMS bar was used (0.5 mm phase) was applied for 1 h 45 °C. After the extraction the stir bar was thermally desorbed at 40 °C, increasing the temperature at 60 °C/min to 280 °C (hold 3 min).



Keynotes

1. Chemical markers of rancid fish have been identified.
2. Instrumental methods that can replace expensive human taste panels are needed by the fish oil industry.
3. Headspace SPME-GC is the most promising technological alternative.
4. The sensitivity and selectivity need improvement for some applications.

microencapsulated fish oil,¹⁸ although quantification was not attempted. For the microencapsulated fish oil, Jonsdottir et al. selected hexanal, 2-nonenal and 2,4-decadienals as quality indicators and found that coating mixtures of caseinate and lactose were superior to sucrose and maltodextrin.

Headspace SPME was also used by Jimenez-Alvarez et al.¹⁹ for the analysis of fish oil-enriched milk emulsions and by Duflos et al. to determine volatile compounds to characterize fish spoilage.²⁰ Unfortunately, no DLs were reported. Iglesias et al. developed an SPME method to determine the volatiles derived from oxidized fish oil-enriched food product.²¹ They reported that the 75 µm carboxen/polydimethylsiloxane (PDMS) fibre coating were superior to both 65 µm PDMS/divinylbenzene (DVB) and 65 µm carbowax/DVB regarding extraction efficiency. The recoveries were good and ranged from 80–120%, whereas the relative standard deviations (RSDs) were found to be below 10%. Optimal extraction conditions for 16 target compounds could be obtained using 0.5 g of sample, 30 min extraction time at an extraction temperature of 60 °C.

Method detection limits (DLs) are very important in this type of analysis because of the low flavour threshold levels of fish oil breakdown products. According to Macfarlane, 1-penten-3-one smells and tastes plastic at 10 ng/g, whereas (E,E)-2,4-heptadienal tastes burning/rancid at 10 ng/g. Iglesias investigated the detection limits for 16 compounds and they were found to be in the low ng/g to high pg/g range depending on the food matrix. For 1-penten-3-one and (E,E)-2,4-heptadienal the limits of detection were 0.22 ng/g and 0.27 ng/g in milk respectively and 0.48 ng/g and 1.84 ng/g in mayonnaise respectively, which should be sufficient for quantification.

Unfortunately, Iglesias did not determine the DLs for the higher boiling ketones and aldehydes, such as 2,6-nonadienal and 1,5-octadiene-3-one, which both have a taste threshold level as low as 10 ng/g. 2,5-nonadienal has an even lower level and it tastes rancid at 1 ng/g. Sensitive measurements of nonadienals are also required to use the FAST index and to use the markers of lipid oxidation, (E,Z)-2,6-nonadienal and 1-pentene-3-one, previously proposed by Jacobsen et al. To ensure that the limits of detection are low enough to maintain precise quantification the SPME method should be performed with maximum sensitivity for the compounds of interest. In SPME, a common way to increase the sensitivity is to increase the extraction phase volume as it has been shown that the amount of semi-volatiles extracted by absorption in a three-phase system (liquid sample/headspace/extraction phase) is proportional to the volume of the extraction phase.²²

Sensitivity improvements have been demonstrated by a coated stir bar²³ and a coated multifibre method,²⁴ or by using other SPME methods with thick coatings.¹ However, increasing the coating thickness increases the equilibration time, resulting in a long analysis time. Alleviation of this limitation can be compensated by increasing the surface area and volume of the extraction phase.

Practical application of this method has been demonstrated previously using a thin sheet of PDMS to extract volatile and semi-volatile environmental pollutants from water.²⁵ An increase in sensitivity can be obtained using headspace sorptive extraction (HSE) compared with SPME to determine 2,6-nonadienal in tuna oil.²⁶ In Figure 1, the mass chromatograms are compared showing that HSE (a) results in a higher signal-to-noise ratio than SPME (b), but at the same time increases the

complexity of the chromatogram. To successfully monitor the volatile decomposition products there is a need for a method that provides both sufficient detection limits and at the same time provides selectivity.

Conclusions

SPME is sensitive enough for the accurate and reproducible determination of a range of volatiles in fish oil. Whether SPME is suitable to determine nonadienals and higher boiling compounds is not clear at the moment. There is a need for more research into microextraction techniques that can provide the same advantages as SPME with improved sensitivity and selectivity. The marine oil industry is constantly exploring novel sources of omega-3 fatty acids.

Recently, there has been a growing interest for polar lipids extracted from sources rich in marine phospholipids, such as fish roe and krill. These oils are highly attractive because of the alleged improvement in bioavailability of the bioactive omega-3 fatty acids. However, the lipid extracts are more complex than traditional fish oils as they comprise of a mixture of neutral and polar fat, carotenoids, as well as volatile protein degradation compounds, resulting in a highly complex headspace, which poses an even greater analytical challenge. To provide the consumer market with marine-based products from these novel sources with superior quality to enhance health, the instrumental technology needs to be developed as well. Methods capable of providing high sensitivity and high selectivity for volatiles and semi-volatiles will be required in the future. Furthermore, for the marine oil industry to adopt these methods it is essential that the methods are robust, reproducible and easily automated.

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