Optimizing Headspace Sampling Temperature and Time for Analysis of Volatile Oxidation Products in Fish Oil

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ABSTRACT: Headspace-gas chromatography (HS-GC), based on adsorption to Tenax GR®, thermal desorption and GC, has been used for analysis of volatiles in fish oil. To optimize sampling conditions, the effect of heating the fish oil at various temperatures and times was evaluated from anisidine values (AV) and HS-GC. AV indicated sample degradations at 90°C but only small alterations between 60 and 75°C. HS-GC showed increasing response with temperature and time. Purging at 75°C for 45 min was selected as the preferred sampling condition for oxidized fish oil.
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KEY WORDS: Fish oil, headspace sampling, HS-GC, lipid oxidation, thermal degradation, volatiles.

Owing to its high content of polyunsaturated fatty acids, especially docosahexaenoic acid and eicosapentaenoic acid, fish oil is highly susceptible to lipid oxidation. During storage of fish oil, this can lead to the formation of a large number of volatile lipid oxidation products, which include a group of volatile compounds with intense odors and flavors (1–3).

Dynamic headspace sampling, coupled to gas chromatography (GC), is widely used for the isolation and analysis of volatile compounds in foods. This method has also been applied in a number of studies for investigations of the composition of volatile oxidation products in fish oil to identify the compounds responsible for off-flavors (2,4,5). According to these studies, the majority of the volatiles of importance to the aroma of oxidized fish oil are carbonyls with carbon chainlengths between 4 and 10 carbon atoms. Owing to the hydrophobic nature of these volatiles, the vapor pressures of the majority of the volatiles are lower when they are dissolved in fish oil than when dissolved in aqueous products (6).

To achieve a sufficient response in headspace analysis of oil products, more vigorous sampling conditions must be applied than when sampling from aqueous materials. Such conditions often include stripping of the oil instead of purging. Also large volumes of sampling gas may be used, necessitating an efficient trapping technique, e.g., cryotrapping or adsorbent trapping.

According to the integrated Clausius-Clapeyron equation (Equation 1),

$$\ln(p_{i1}/p_{i0}) = \Delta H_{ivap}/R\,(1/T_0 - 1/T_1) \qquad [1]$$

the vapor pressure p_i of a solute i increases with temperature T, i.e., the headspace sampling efficiency can be improved by increasing the sampling temperature. According to Nelson and Hoff (6), an increase in sampling temperature especially benefits the sampling efficiency of high-boiling compounds. However, at higher temperatures, the degradation of lipid hydroperoxides is accelerated, thus involving a risk of alteration of the composition and concentration of volatiles during sampling (3). The object of this study therefore was to find a compromise between conditions for a high sampling efficiency and a low extent of sample alterations.

EXPERIMENTAL PROCEDURES

Apparatus. Glass tubes for headspace sampling consisted of the sampling tube (19 mm i.d., length 118 mm), fitted with a ground tapered joint, on which was placed a washing bottle head (gas inlet tube 115 mm in length ending with an opening of 0.6 mm i.d.). The outlet (6 mm o.d., 4 mm i.d.) from the bottle head was connected [stainless-steel fitting (Swagelock Co., Solon, OH) and Teflon® ferrule] to a Perkin-Elmer (Norwalk, CT) stainless-steel adsorbent trapping tube, packed with 225 mg 60–80 mesh Tenax GR® (Chrompack, Middelburg, The Netherlands). The tubes were closed with Teflon® caps until use. Before each use of the sampling tubes, they were conditioned by thermal desorption in the ATD400 desorber (see below) for three times 12 min at 325°C. A thermostat-regulated water bath was used to control the sampling temperature. A Rotameter (Porter Instrument Co., Hatfield, PA) was used to monitor the nitrogen flow during sampling. A soap-bubble flowmeter was used in the beginning of the sampling period to ascertain that no leaks were present (comparison of flow at adsorbent tube outlet with Rotameter inlet flow).

A Perkin-Elmer ATD400 automatic thermal desorber with a Tenax TA®-packed cold trap, connected to a Hewlett-

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Packard (Avondale, PA) 5890A gas chromatograph with flame-ionization detector was used for thermal desorption and subsequent GC. The gas chromatograph was equipped with a DB1701 capillary column (J&W Scientific, Folsom, CA) of 1-µm film thickness (0.32 mm i.d., 30-m length). Helium was used as carrier gas (1.8 mL/min) in the temperature-programmed GC separation (initial temperature: 32°C for 3 min; increasing by 3°C/min to 140°C, then by 5°C/min to 170°C, and finally by 10°C/min to 240°C, which was maintained for 8 min). Softron PC Integration Pack (Kontron Instruments, Milano, Italy) was used for relative quantitation.

Materials. Refined and deodorized sand eel oil, provided by the Project Fish Oil Group, Technical University of Denmark (Lyngby, Denmark), was moderately oxidized in the laboratory (20–25°C) in an open glass vessel under stirring for 1 wk [anisidine value (AV) = 3.2]. Nitrogen (99.995%) and helium (99.9995%) were from Hydrogas (Fredericia, Denmark). 1-Penten-3-one, 2-*t*-pentenal, and 2,4-*t,t*-heptadienal were from Aldrich-Chemie (Steinheim, Germany). *p*-Anisidine was purchased as a 0.25% solution in glacial acetic acid (Bie & Berntsen, Roedovre, Denmark); isooctane (p.a.) was from Merck (Darmstadt, Germany).

Procedures. Volatiles were sampled from 3.8 g fish oil, weighed into a sampling tube. Volatiles were stripped from the oil at a nitrogen flow of 150 mL/min. The volatiles were trapped at ambient temperature in the adsorbent tubes. For GC, the tubes were mounted in the ATD400, set for automatic thermal desorption at 200°C with a helium flow of 40 mL/min. In the ATD400, the thermally desorbed volatiles were cryofocused at –30°C in a Tenax®TA trap. By ballistic heating of the trap to 200˚C, the volatiles were transferred, *via* heated (200°C) fused-silica tubing, into the column of the gas chromatograph. In this transfer, a split ratio of 1:2.8 (column/outlet flow ratio) was used. AV were determined according to the AOCS Official Method (7).

To examine the effect of sampling conditions on AV, 8 g of a moderately oxidized sand eel oil were weighed into each of twelve 15-mL test tubes. Nitrogen was gently blown above the surface for 15 s before the sample tubes were closed with ground-glass stoppers. The oil samples were heated on a water bath according to a complete factorial design at temperatures of 60, 75, and 90°C and for 15, 30, or 45 min. An additional three samples were heated at 75°C for 30 min to determine variations between samples. AV of the heat-treated and subsequently chilled (5°C) oil samples were determined in duplicates.

To examine the effect on sampling efficiency, the volatiles of identical samples of moderately oxidized fish oil were sampled at 60, 75, or 90°C for 30 or 45 min. The trapped volatiles were thermally desorbed and analyzed by GC. Peak areas were used for relative quantitation. Identifications of volatiles were made by GC–mass spectrometry, supported by comparison of linear retention indices of standard compounds when available, and by odor characteristics described in the literature (3,4).

RESULTS AND DISCUSSION

Influence on the AV. The AV is a sensitive measure of the oxidative status of fish oil (8–10). Because AV is a nonspecific measure of carbonyl compounds in oxidized lipids, it is likely to correlate with the concentration of volatile oxidation products. Therefore, AV was used as an indicator for the effect of various thermal exposures of oxidized fish oil, simulating headspace sampling at various times and temperatures.

In Figure 1, AV is plotted against heating time. There are significant effects (95% level) of heating temperature, heating time, and interactions between these two factors. As seen in Figure 1, increasing temperature from 75 to 90°C has a large effect on the rate of AV increase with time. The effect of increasing the temperature from 60 to 75°C is much smaller, although still statistically significant (95% level). This confirms that the oxidized oil will undergo chemical changes at high sampling temperatures. The rates of AV development are taken as an indication that 90°C is too high for volatile sampling from an oxidized oil. The observed AV increase may be assumed to be due to thermally induced breakdown of lipid hydroperoxides (10–12). The relatively modest difference in AV development, seen when heating at 75°C in comparison with 60°C, indicates that thermal sample degradation in this moderately oxidized fish oil is of minor importance below 75°C. Preliminary experiments have shown that sampling temperatures below 60°C are not applicable for this purpose, because compounds of longer chainlength (e.g., decanal) cannot be collected at these sampling temperatures (data not shown).

Influence on sampling efficiency. The GC peak areas obtained at different sampling conditions of selected and representative volatile compounds in a moderately oxidized fish oil are shown in Figure 2. For the majority of these compounds, the peak areas increase markedly with sampling temperature and time. This may be a result of the combined effect of increased liberation of volatiles at the higher sampling temperature and a higher concentration of volatiles in the sample due to degradation of hydroperoxides.

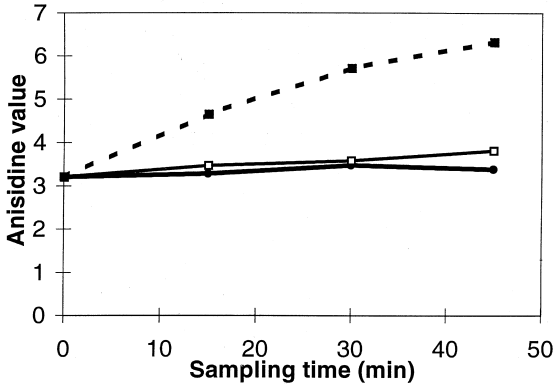

FIG. 1. Anisidine values plotted against duration (min) of thermal exposure at 60°C (●), 75°C (□), and 90°C (■).

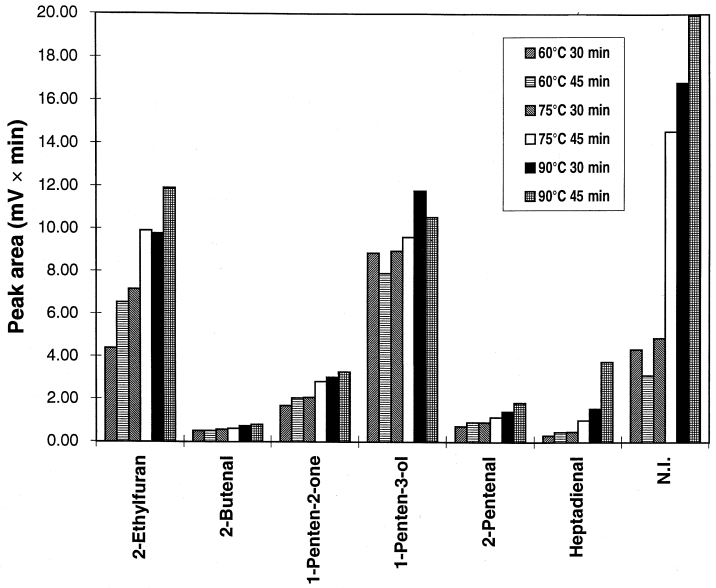

FIG. 2. Peak areas obtained by headspace-gas chromatography–flame-ionization detection of selected volatiles in moderately oxidized fish oil at different sampling times and temperatures (N.I.: compound not identified).

Optimization of sampling conditions. The high rate of AV increase observed during heating of the fish oil at 90°C indicates that this temperature is not suited for analysis of volatile oxidation products in fish oil (Fig. 1). Comparison of time and temperature effects on sampling efficiency (Fig. 2) demonstrates that sampling at 75°C for 45 min results in a response that is higher than obtained by sampling at 60°C. According to Figure 1, the AV indicate that the extent of sample degradation is only influenced to a limited degree when raising the sampling temperature from 60 to 75°C. Sampling at 75°C for 45 min thus seems to be the sampling condition for headspace analysis of fish oil that optimizes the criteria of a high sensitivity combined with a low risk of sample alterations.

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