Determination of Aldehydes in Fish by High-Performance Liquid Chromatography

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Abstract

A new analytical method to determine trace volatile aldehydes isolated from the headspace of fish meat at room temperature by high-performance liquid chromatography (HPLC) in the form of 2,4-dinitrophenylhydrazone (DNPHo) derivatives has been developed. Aliquots (50 g) of the fish purée were introduced into a 500-mL glass recipient and were purged with N_2 for 40 min through two SEP-PAK C18 cartridges (connected in series) coated with an acid solution of 2,4-dinitrophenylhydrazine. The cartridges were then eluted with acetonitrile (2 mL) and the 2,4-DNPHo formed was quantitated by HPLC–UV analysis using a Zorbax C18 column. The isolated compounds from the dynamic headspace sampling of four kinds of fish species were saturated aldehydes, formaldehyde, acetaldehyde, propanal, butanal, pentanal, and hexanal. Under optimized conditions the detection limits of the HPLC method were in the range of 0.75 nmol/g (formaldehyde) to 2.19 nmol/g (hexanal). The calibration curves were linear in the concentration range from 1.3 nmol/mL to 12.5 nmol/mL. Propanal and acetaldehyde were the major carbonyl compounds identified (ranging from 3.9 nmol/g and 10 nmol/g). This study has revealed the widespread occurrence of formaldehyde, acetaldehyde, propanal, butanal, pentanal, and hexanal in fish meat.

Introduction

Volatile aldehydes (including saturated, α,β-monounsaturated, and polyunsaturated) are formed as secondary products from the lipid oxidation of polyunsaturated fatty acid either by a chemical or enzymatic process in food systems such as fish meat (1). These volatile compounds at low concentration can contribute to the fresh aroma of various species of fish, but a higher concentration may convey changes in flavor, color, and texture (2). For example, a green and grassy off-odor in fish may be attributed to high levels of hexanal (3). Indeed, fish tissue is an important medium to study because of its importance as a food source and an indicator of the overall quality of an environment (4).

Saturated aldehydes are believed to be one of the main contributors of food rancidity that represents the major cause of a loss of nutritional quality in food (2). Moreover, in the last two decades aldehydes have received a great deal of attention because of their recognized adverse health effects. In this way, exposure to formaldehyde and acetaldehyde results in toxic effects such as irritation to the eyes and respiratory tract, nausea, headaches, and thirstiness (5,6). Besides the environmental and health importance of these compounds, the knowledge of their concentrations in various kinds of food and beverages is sketchy at best.

In regards to the specific reaction between carbonyl compounds and nucleophiles (including hydrazine derivatives), a common analytical procedure employed in the speciation and quantitation of carbonyl compounds involves a reaction with an acidic solution of 2,4-dinitrophenylhydrazine (DNPHi) to form the corresponding 2,4-dinitrophenylhydrazone (DNPHo) (Figure 1). The hydrazones thus formed are then separated by gas chromatography (GC) or high-performance liquid chromatography (HPLC). Because of the difficulties associated with the GC analysis of DNPHo, the use of HPLC has increased (7–15).

Indeed, the direct reaction of fish tissue with an acid solution of 2,4-DNPHi is not useful for food analysis because the derivatization reaction requires a strongly acidic medium that can cause undesirable reactions in the sample (16). The main objective of this work was to develop a sampling method that makes use of 2,4-DNPHi in the analysis of aldehydes in fish. The species *Ocyurus sp.*, *Lutjanus sp.*, *Rachycentron sp.*, and *Rhomboplites sp.* were studied.

Experimental

Reagents and standards

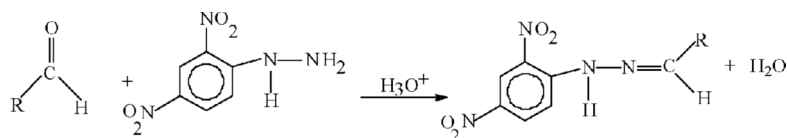Acetonitrile and ethanol (HPLC grade) were obtained from

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Figure 1. Reaction between aldehyde and 2,4-DNPHi producing 2,4-DNPHo: (R) aril or alquil.

Aldrich (Milwaukee, WI) and Merck (Darmstadt, Germany). Purified water was obtained by distillation and filtration through an E-pure Alltech (Deerfield, IL) system. The other reagents were of analytical grade.

Preparation of the 2,4-DNPHi solution

The 2,4-DNPHi solution (pH = 1.85) was prepared at 0.05% (w/v) in acetonitrile–H_2O–H_3PO_4 (20:79:1, v/v/v) and then purified by liquid–liquid discontinued extraction with CCl_4. It was stored at 4°C in total darkness. The purity of the solution was verified by HPLC–UV analysis. A more detailed account of reagent preparation can be found elsewhere (14).

2,4-DNPHo standards

The DNPHo derivatives formaldehyde, acetaldehyde, propanal, butanal, pentanal, and hexanal were synthesized from the reaction of 2,4-DNPHi with corresponding carbonyl compounds according to the methods of Shriner et al. (16) and purified by recrystallization from ethanol. The purities of 2,4-DNPHo were verified by the comparison of their melting points with the literature.

Preparation of the carbonyl–DNPHo calibration solution

A stock solution (20 µg/mL) of a mixture of hydrazone derivatives was prepared by first weighing each hydrazone derivative and then dissolving them in acetronitrile. The standard solutions were prepared from the stock solution in concentration ranges from 0.05 µg/mL to 1.20 µg/mL. They were stored at 4°C in total darkness.

Preparation of DNPHi–injected cartridges (SEP-PAK C18)

The cartridges were washed with 2 mL of acetonitrile and injected with 4 mL of 2,4-DNPHi solution (0.05%). Excess liquid was blown out of the cartridges with N_2 (free of carbonyl compounds). The cartridges were wrapped in aluminum foil and dried in a dessicator and then stored in a refrigerator at 4°C.

Sample preparation

Fresh fish (*Ocyurus sp.*, *Lutjanus sp.*, *Rachycentron sp.*, and

Figure 2. Dynamic headspace sampling: N_2 (flow rate = 0.369 L/min), 1; SEP-PAK C18 cartridges coated with 2,4-DNPHi, 2 and 3; 500-mL glass recipient, 4; and sample, 5.

Rhomboplites sp.) were purchased at local markets. After their arrival at the laboratory, the fish were washed sequentially with distilled water and a saturated NaCl solution. Then, their meat was transferred to a food processor and blended into a fine purée. The purée was stored in a sealed polyethylene bag at –15°C until analyzed.

Method

Dynamic headspace sampling (purge and trap)

Aliquots (50 g) of the grated fish samples were introduced into a 500-mL glass recipient and purged with N_2 for 40 min through two SEP-PAK C18 cartridges (Waters Corporation, Milford, MA) connected in series. The cartridges were coated with an acid solution of 2,4-DNPHi (Figure 2). Under these conditions all of the six carbonyl compound studied were trapped on the first cartridge. Then, the treated compounds were eluted with acetonitrile (2 mL). The 2,4-DNPHo that formed was separated and quantitated by HPLC–UV analysis.

Compounds separation

The derivatives were separated with a Zorbax (Rockland Technologies, Chadds Ford, PA) ODS 5-µm column (4.6 mm × 25 cm) using an acetronitrile–water mixture (57:47, v/v) as a mobile phase at a flow rate of 1.5 mL/min. Also used was a Varian (Walnut Creek, CA) liquid chromatography model 2510 equipped with a Rheodyne (Cotati, CA) injector with a 10-µL sample loop. Compound detection was made by absorbance (Varian UV–vis detector model 2550) at 365 nm (AUFS = 0.04).

Results and Discussion

The detection limit that an analytical procedure may achieve greatly depends on the reagent blank quality. Even with successive recrystallizations the DNPH showed contamination by formaldehyde and acetaldehyde DNPHo. The new purification procedure described in this study resulted in a blank level for all aldehydes as low as 1–3 nmol/g and thus a detection limit of the HPLC method (signal-to-noise equal to 3 based on peak height) in the range of 0.75 nmol/g to 2.19 nmol/g for formaldehyde and hexanal, respectively (Table I).

Different concentrations of formaldehyde, acetaldehyde, propanal, butanal, pentanal, and hexanal DNPHo solutions in the concentration range of 1.0 nmol/mL to 12.5 nmol/mL (*n* = 6
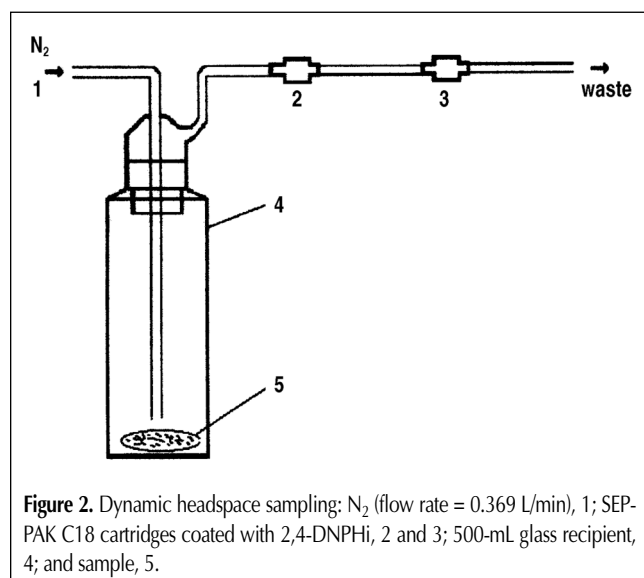
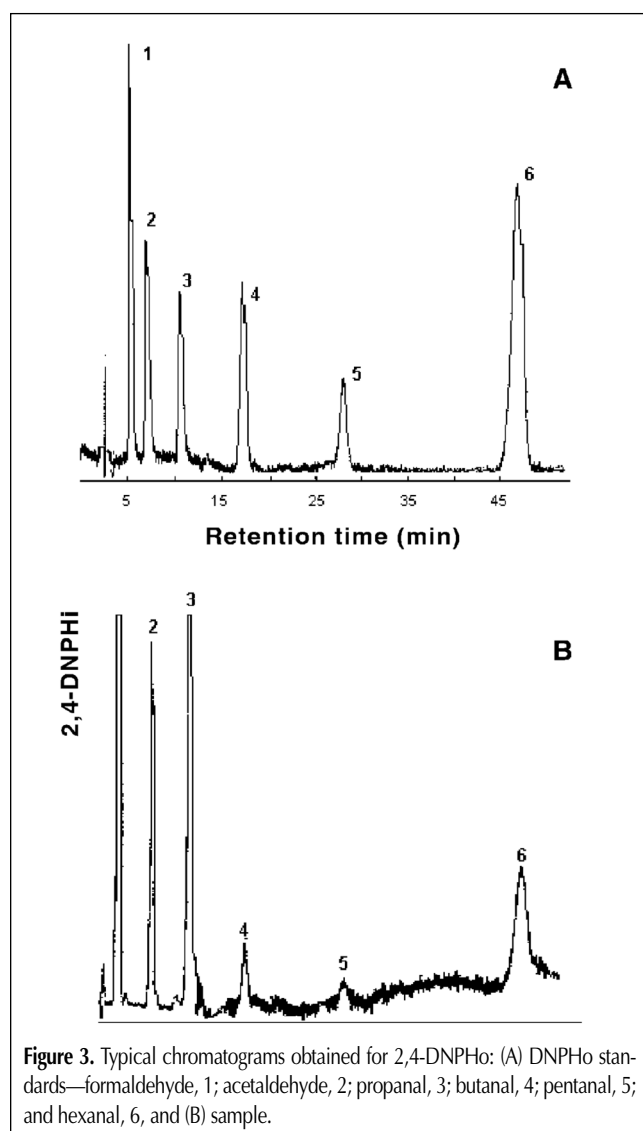| Table I. Detection Limit of the HPLC Method*	
Aldehyde	**Detection limit (nmol/mg)**
Formaldehyde	0.75
Acetaldehyde	0.86
Propanal	1.4
Butanal	1.7
Pentanal	1.9
Hexanal	2.19
* Signal-to-noise equals 3 based on peak height.	

points) were injected into the HPLC system and the peak heights obtained were plotted versus concentration. The calibration curves showed good linearity (as shown in the Table II). The HPLC determination was completed in approximately 48 min (Figure 3).

Many of the methods previously employed for the simultaneous determination of trace levels of volatile aldehydes in food samples

were based on directly treating the sample with the derivatization reagent (18,19). This pH-dependent reaction proceeds by a multi-step mechanism in which the rate-limiting step involves the addition of the weak nucleophile 2,4-DNPHi to the protonated carbonyl (20). Because the derivatization reaction requires a strongly acidic medium, it can cause undesirable reactions in the sample (for example, the decomposition of the trimethylamine oxide present in fish meat into formaldehyde and trimethyamine is possible). However, the direct reaction of the fish sample with an acidic solution of 2,4-DNPHi at pH 1.5–2.0 and room temperature allows for the quantitation of only the most electrophilic aldehydes, particularly formaldehyde and acetaldehyde. Nevertheless, it should be noted that the results obtained could be minimally consistent because after 20 min of direct reaction the pH increased to 5.0 and the determined concentrations of formaldehyde and acetaldehyde decreased.

Table II. Calibration Curves for the HPLC Determination of Formaldehyde, Acetaldehyde, Propanal, Butanal, Pentanal, and Hexanal as DNPHo Derivatives*

Aldehyde	Peak height	Intercept	Coefficient of determination
Formaldehyde	87.347	–0.5911	0.9988
Acetaldehyde	47.858	–0.0551	0.9999
Propanal	24.629	0.7011	0.9999
Butanal	23.661	–2.0054	0.9989
Pentanal	24.564	–2.1982	0.9999
Hexanal	10.117	–0.6888	0.9999

* The peak height is equal to the sum of the slope times the concentration (mg/L) and the intercept.

Conclusion

The new methodology described using dynamic headspace sampling involved the isolation of volatile aldehydes from a fish sample followed by purging with N_2 (Figure 2) and chemical adsorption onto two SEP-PAK C18 cartridges coated with an acid solution of 2,4-DNPHi. The desorption of the hydrazones formed was made possible by solvent extraction with acetonitrile. The use of SEP-PAK C18 cartridges avoided the acid effect that may cause a chemical change in the biological samples.

Six carbonyl compounds were quantitated using this new methodology. The concentration ranges (nmol/g) of formaldehyde, acetaldehyde, propanal, butanal, pentanal, and hexanal determined in the fish samples are listed in Table III. Acetaldehyde and propanal were the most abundant carbonyl compounds found in all the species. Acetaldehyde at a dilute concentration gives a pleasant fruit aroma (21) and is probably derived from the oxidation of fish lipid ω-3 fatty acids (22). Propanal originates from the breakdown of ω-3 fatty acid peroxide (16).

There are a number advantages of this new analytical procedure. Primarily, the low blank level obtained for formaldehyde, acetaldehyde, propanal, butanal, pentanal, and hexanal resulted in low detection limits. In addition, the method is simpler than

Figure 3. Typical chromatograms obtained for 2,4-DNPHo: (A) DNPHo standards—formaldehyde, 1; acetaldehyde, 2; propanal, 3; butanal, 4; pentanal, 5; and hexanal, 6, and (B) sample.

Table III. Concentrations in Nanomoles per Gram of Formaldehyde, Acetaldehyde, Propanal, Butanal, Pentanal, and Hexanal Determined in Fish from Four Species

Aldehyde	*Ocyurus sp.*	*Rachycentron sp.*	*Lutjanus sp.*	*Rhomboplites sp.*
Formaldehyde	2.3–2.8	n.d.*–2.0	2.2–2.4	n.d.–2.53
Acetaldehyde	8.0–8.9	0.95–2.3	7.9–10	5.7–6.8
Propanal	0.69–1.7	0.86–2.9	2.6–3.6	2.9–3.9
Butanal	n.d.	0.30–0.39	n.d.–0.25	n.d.
Pentanal	n.d.	0.17–0.23	n.d.–2.6	n.d.
Hexanal	n.d.–0.55	n.d.–0.60	0.57–0.70	n.d.–0.38

* n.d., could not be determined.

the methods described in the literature and very little sample preparation is required.

Acknowledgments

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