Identification of Major High-Boiling Volatile Compounds Produced During Refrigerated Storage of Haddock Fillets

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The two major high-boiling volatile compounds produced during refrigerated storage of haddock fillets were found by gas chromatography and mass spectroscopy to be phenethyl alcohol and phenol.

The major genera of bacteria responsible for the spoilage of refrigerated fish have been studied extensively. Little work, however, has been reported on the major high-boiling metabolites produced in fish tissue during refrigerated storage. Wong et al. (3) reported the formation of benzene, toluene, and several ketones in cod held at 0°C. This study documents the formation of phenethyl alcohol and phenol as the major high-boiling volatile components produced in haddock fillets stored at 2°C.

MATERIALS AND METHODS

Isolation of volatile components from haddock. A 30-g amount of fish tissue was placed into a stainless-steel centrifuge tube (50 ml capacity), 15 ml of Mazola corn oil was added, and the mixture was homogenized with a glass rod. After thoroughly blending in this manner, the oil phase was separated by centrifugation at 17,000 × g for 20 min. A 10-ml volume of the oil was then used for molecular distillation (2).

The sample container was immersed in an oil bath at 80°C, and the oil sample was stirred continuously by a Teflon-coated magnetic stirring bar. The volatiles were collected from the oil by distillation under a vacuum of approximately 10⁻³ torr, and condensed onto a liquid nitrogen-cooled cold finger (2). After 2 h of distillation, the cold finger was removed, and about 10 ml of anhydrous diethyl ether was used to rinse the collecting surface. Prior to gas chromatography analysis, the ether was concentrated to 20 μl in a vial with a gentle flow of nitrogen. A volume of 2 μl of the solution was used for injection into gas chromatography columns.

Separation and identification of the major volatile components. A Perkin-Elmer model 881 dual-column gas chromatograph equipped with a flame ionization detector was used with a 1 mV full-scale Honeywell model W recorder for analysis of collected volatiles. After various columns were tested, a 12-ft (ca. 3.7 m) column packed with Carbowax 20M was used for initial separation of the volatile components.

Column temperature was maintained at 145°C, and prepurified nitrogen (Airco) was used as the carrier gas.

Using a combined gas chromatograph and mass spectrometer unit, the mass spectra of the major high-boiling volatile components were obtained. The effluent from an Aerograph 1200 gas chromatograph was admitted via a heated line to a Bieman Helium separator and then to the ion source of an Hitachi-Perkin Elmer RMU-6A mass spectrometer. The identification of volatile compounds was verified by comparing their mass spectra and gas chromatography retention times on two different 12-foot columns (Carbowax 20M and diethylene glycol succinate).

Bacterial count on haddock fillet. The viable bacterial count on the haddock fillet used above was determined on pour plates of Trypticase soy agar without dextrose (BBL) incubated at 2°C for 6 days.

RESULTS AND DISCUSSION

Increase of volatile components during refrigerated storage of haddock. A market fresh haddock fillet of high organoleptic quality was found to contain few high-boiling volatile compounds at zero time storage (Fig. 1). After 5 days of storage, a number of high-boiling volatile peaks were present in relatively low concentration. On day 9 of storage, a major peak, designated peak 40, was present. On day 15, coincident with the development of a maximal bacterial population on the tissue (Fig. 2), peak 40 had decreased notably and a third peak, designated peak 44, predominated. On day 20 of storage, peak 40 was only barely detectable, whereas peak 44 had greatly increased.

Identification of the major high-boiling volatile compounds. Peaks 40 and 44 were identified by mass spectroscopy as phenethyl alcohol and phenol, respectively (Fig. 3 and 4). Authentic phenethyl alcohol (C₆H₅CH₂CH₂OH, Eastman Organic Chemicals) and phenol (Fisher Scientific Co.) were used for verification. Both the mass spectral data and gas
Fig. 1. Gas chromatographic profiles of volatile components formed on a haddock fillet stored at 2°C.

Fig. 2. Viable bacterial count on haddock fillet stored at 2°C.

chromatography retention times of these two compounds confirmed the experimental results from fish extracts. At an isothermal column temperature of 145°C, phenethyl alcohol had a retention time of 25 min on a 12-foot Carbowax 20M column and 11 min on a 12-foot diethylene glycol succinate column. Phenol had a retention time of 49 min on the Carbowax column and 13 min on the diethylene glycol succinate column. The genus Achromobacter was found to be responsible for phenethyl alcohol production and is the subject of an accompanying report (1).

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