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Headspace solid phase microextraction-gas chromatography for the determination of trihalomethanes in fish

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ABSTRACT

The aim of this work is to develop a method for determining trihalomethanes (THMs) in fish samples. The proposed method uses solid phase microextraction and gas chromatography with an electron capture detector. Factors such as temperature, extraction time and type of fiber were assessed to maximize the performance of the extraction technique. The performance of the method was evaluated using selectivity, linearity, precision, accuracy and limits of detection (LOD) and quantification (LOQ). The new method allows analysis of THMs with appropriate selectivity and linearity, with coefficient of correlation >0.98. The LOD and LOQ of the analytes of interest are from 0.11 to 0.35 μg kg^{-1} and 0.35 to 1.18 μg kg^{-1}, respectively. In addition, the relative standard deviation (RSD) was between 1.6 and 8%, and the relative recovery was between 76 and 113%. The optimized and validated method was applied to fish samples purchased from the Viçosa (MG) local market. At least three of the THMs of interest were detected in most of the analyzed fish samples with maximum values for the concentration of chloroform, bromodichloromethane and bromoform at 8.33, 0.42 and 2.41 μg kg^{-1}, respectively.

1. Introduction

In the fishing industry, the expression "quality of fish" is often related to species, marketable size and commercial value. Fish considered of low quality for a fish processor could be too small or in bad condition due to incorrect processing or the presence of wounds or spots on their back, resulting in low prices and thus low profits. Very often, however, quality means freshness and good appearance, and it is related to the degree of deterioration inherent to fish [1].

To insure the quality of fish, the slaughtering and cleaning processes are very important. The processing is designed to prevent the action of digestive enzymes before evisceration, which affect parameters such as pH, total volatile nitrogenous bases, shear force, color and the sensory attributes of the fish [2]. For initial processing, the fish is immediately washed to remove mucus, which is composed of glycoproteins released by the skin glands. This washing procedure associated with hyperchlorination (10 mg L^{-1}), also reduces the microbiota on the fish surface [3].

After slaughtering, the fish scales and guts are removed. Then, fish are washed with chlorinated drinking water to remove adhered residues. At this point, the fish are clean and could be packed and cooled or frozen for commercialization or continue to be filleted. Since fish deteriorates easily, ice must be used for cooling. Ice cubes (or any other shape) with a maximum volume of 1 cm^3 are used, in the ratio ice:fish of 1:1 [4]. After filleting, another step of washing with treated water is necessary.

For commercialization, the fish can be kept fresh, cooled or frozen. "Fresh" fish are consumed without having undergone additional conservation process, except for being kept on ice action. "Cooled" fish are kept properly packaged in ice and at temperatures between −0.5 and −2 °C, and "frozen" fish are kept frozen with temperatures not to exceed −25 °C [5].

For fish stored under refrigeration, microbial proliferation has been identified as the main cause of deterioration [6]. An alternative method that has been used in washing and storing the fish is the use of chlorinated water and ice. Scherer et al. [6] observed that the use of chlorinated water and ice effectively reduces the psychotropic, mesophilic and aerobic microorganisms in grass carp meat, increasing the shelf life of the whole fish stored under refrigeration by approximately three days.

However, some by-products, as trihalomethanes (THMs), may be formed during chlorination process. The chloroform (CF), dichlorobromomethane (DCBM) chlorodibromomethane (CDBM) and bromoform (BF) are the most important THMs. THMs are mutagenic, cause various types of cancer, and may take more than thirty years to degrade in the environment [7].

The World Health Organization (WHO) recommends that the highest levels of chloroform, dichlorobromomethane,

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chlorodibromomethane and bromoform allowed in drinking water are 200, 60, 100 and 100 μg L^{-1}, respectively [8]. The maximum contaminant level allowed by Brazilian legislation for the total concentration of THMs in water is 100 μg L^{-1}, which corresponds to the sum of the concentrations of the four major compounds: chloroform, dichlorobromomethane, chlorodibromomethane and bromoform.

Some studies from England testing several kinds of foods have found the presence of chloroform at the following concentrations: 1.4 to 33 μg kg^{-1} in dairy products; 1 to 4 mg kg^{-1} in meat; 2 to 10 mg kg^{-1} in vegetable oils; 0.4 to 18 mg L^{-1} in beverages; and 2 to 18 mg kg^{-1} in fruits and vegetables [9]. In fish meat, the concentration of CF was from 4 to 52 μg kg^{-1}; in chicken meat, between 2 and 76 μg kg^{-1}; and in pork meat, between 2 and 68 μg kg^{-1} of CF and 11 μg kg^{-1} of DCBM [10–12].

Since fish is a product with rapid deterioration and quality loss, the use of preservatives and methods to increase its shelf life, especially compared to fresh fish, is essential for the success of aquaculture activity. However, monitoring the by-products from the employed methods is very important. Thus, a headspace solid phase microextraction (HS/SPME) with gas chromatography (GC) method was optimized and validated to simultaneously determinate the four major THMs in fish samples.

2. Material and methods

2.1. Chemicals and standard solutions

All reagents used were of analytical grade. The stock solutions of trihalomethanes in methanol (Merck, Darmstad, Germany), were prepared from standard mixture obtained from Supelco, Inc. (Bellefort, PA, USA), containing chloroform, bromodichloromethane, dibromochloromethane and bromoform at a concentration of 2000 mg mL^{-1}. The solutions were stored at −18 °C. The working solutions were prepared daily from the stock solutions.

2.2. Samples

For procedure optimization and method validation, fish (Nile tilapia fillets) that were slaughtered and sanitized with water (free of chlorine) was purchased from subsistence farms in Viçosa/MG.

The optimized and validated method was applied to 13 samples of fish fillets from different size fish farms, small, medium and large, which were bought at the local market in Viçosa/MG. Extractions and chromatographic analysis, were carried out for different storage periods.

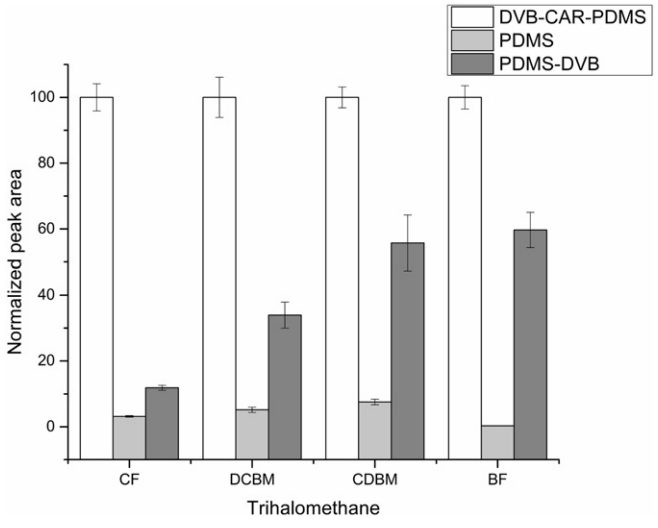

Fig. 1. The influence of fiber type on the chromatographic peak areas of THMs extracted from fish samples at 1 μg kg^{-1}. Sample mass, 2 g; extraction time, 15 min. (CF = chloroform, DCBM = dichlorobromomethane, CDBM = chlorodibromomethane and BF = bromoform).

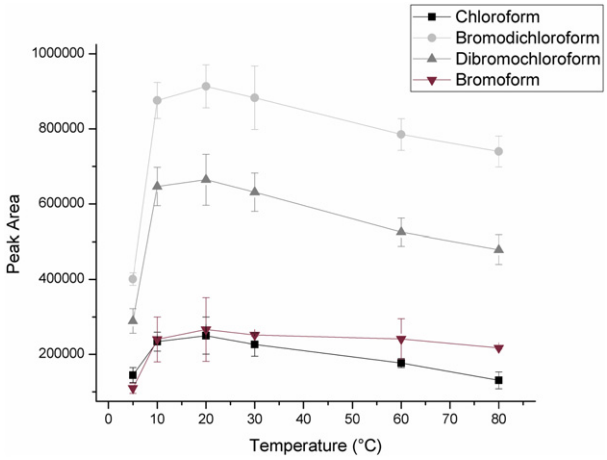

Fig. 2. The influence of temperature on the chromatographic peak areas of THMs extracted from fish samples at 1 μg kg^{-1}. Sample mass, 2 g; extraction time, 30 min.

2.3. Chromatographic conditions

A gas chromatograph Shimadzu model 2014 with an electron capture detector was used to determine the trihalomethanes. Separations were performed on a Restek RTX-5MS capillary column, with the stationary phase composed of 5% diphenyl and 95% dimethylpolysiloxane (30 m × 0.25 mm i.d. and 0.10 μm thick film). The optimized chromatographic conditions were injector temperature, 200 °C; column oven temperature, 45 °C (2 min); heating ramp of 40 °C, min^{-1} to 100 °C; detector temperature, 300 °C; flow of carrier gas (N_2) of 1.2 mL min^{-1}; and flow division (split) 1:10.

2.4. HS/SPME procedure

Method optimization tests to determine trihalomethanes in fish were made with a manual holder for SPME (Supelco, Bellefort, PA, USA) equipped with a fiber. The THM free fish samples (Nile tilapia fillets) were fortified with the 4 analytes of interest (chloroform, bromodichloromethane, dibromochloromethane and bromoform) at concentrations of 1 μg kg^{-1}.

The optimized method was performed on 2.0 g samples of spiked fish in 20 mL glass flasks equipped with PVC covers and PTFE skiving

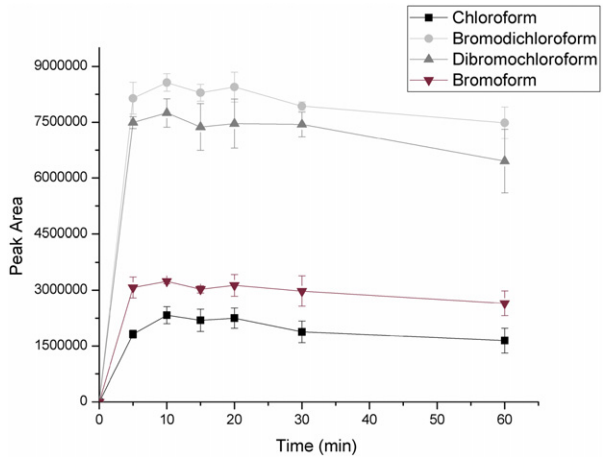

Fig. 3. The influence of exposure time on the chromatographic peak areas of THMs extracted from fish samples at 1 μg kg^{-1}. Sample mass, 2 g; extraction temperature, 30 °C.