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Volatile sulfur compounds in Cheddar cheese determined by headspace solid-phase microextraction and gas chromatograph-pulsed flame photometric detection

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Abstract

The aim of this study was to develop a methodology for the analysis of volatile sulfur compounds (VSCs) in Cheddar cheese. Solid-phase microextraction (SPME) was employed to extract VSCs from the cheese matrix using a CAR-PDMS fiber. This extraction method was combined with gas chromatography–pulsed flame photometric detection (GC–PFPD) to achieve high sensitivity for sulfur compounds. The impact of extraction parameters, including time, temperature and sample size, was evaluated to determine the best conditions to analyze sulfur compounds in Cheddar cheese. Hydrogen sulfide, methanethiol, and dimethyl sulfide were found to constitute the majority of the overall sulfur profile while dimethyl disulfide and dimethyl trisulfide were present in lesser amounts. Artifact formation of volatile sulfur compounds was found to be minimal. Two commercial cheese samples were analyzed and differences in sulfur content were observed. Overall, SPME–GC–PFPD was found to be a highly sensitive technique for the analysis of sulfur compounds in Cheddar cheese.

Keywords: Volatile sulfur compounds; Cheese; Food analysis; Pulsed flame photometric detection

1. Introduction

Volatile sulfur compounds (VSCs), most with low odor thresholds, are considered important flavor contributors to cheeses [1–6]. Methanethiol, hydrogen sulfide, and dimethyl sulfide have all been found to be significant to the flavor of Cheddar cheese [7–11]. The ripening process of Cheddar cheese involves, in part, the decomposition of sulfur-containing amino acids, cysteine and methionine. An increase in the concentration of methanethiol as Cheddar cheese ages has been reported [12] and that it is postulated that other sulfur-containing compounds may also follow a similar trend with aging. Methanethiol not only contributes to Cheddar cheese flavor but also is a precursor for several other sulfur compounds. Once formed, methanethiol can be readily oxidized to create dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide and other sulfur compounds [13,14].

While there is definite evidence that various sulfur compounds are present in Cheddar cheese, their significances to Cheddar cheese flavor are still poorly understood. This contributes to the fact that the volatile sulfur profile of Cheddar cheese highly relies on the specific method used for extraction and/or concentration techniques. Many conventional techniques including solvent extraction, static headspace, and purge-and-trap are not quite suitable for the analysis of VSCs in cheese. Each of these techniques has associated problems when it comes to extracting highly volatile sulfur compounds from cheese matrices including, respectively, the loss of analytes during the concentration stage, particularly compounds with high volatility; insufficient sensitivity for trace components; and great potential of thermal artifact formation [15–17].

Solid-phase microextraction (SPME) can effectively extract and concentrate aroma compounds and also provides

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high sensitivity with minimum artifact formation. With the use of SPME fibers, there is no requirement for organic solvents, and sample preparation can be completed in minimal time. In addition, SPME equipment can be automated. While there are a growing number of available fiber coatings, the Carboxen-polydimethylsiloxane (CAR-PDMS) fiber has repeatedly demonstrated its exceptional ability to extract sulfur compounds including methanethiol and dimethyl sulfide from food [18–25]. The process of concentration with the CAR-PDMS fiber is *adsorption* of small molecules into micro-pores by the Carboxen phase in addition to *absorption* by the PDMS coating [22], lending to its greater capacity for extracting highly volatile, low molecular weight molecules, which includes most VSCs.

Pulsed flame photometric detection (PFPD) is a very sensitive sulfur-specific method of detection that uses a pulsed flame for the generation of flame chemiluminescence. Unlike traditional flame photometric detection (FPD), which uses a continuous flame, the PFPD utilizes low gas rates so that the flame is ignited, propagated and self-terminated 2–4 times per second. Specific elements have their own emission profile: hydrocarbons will complete emission early while sulfur emissions begin at a relatively later time after combustion. Therefore, a timed "gate delay" can selectively allow for only emissions due to sulfur to be integrated, producing a clean chromatogram. This timed "gate delay" greatly improves the sensitivity; the PFPD can detect sulfur-containing compounds at a much lower detection limit than nearly all other methods of detection [26]. The combination of SPME with GC–PFPD greatly enhances the ability to successfully extract and detect VSCs in cheese at low concentrations. The objective of this study was to develop a methodology using SPME–GC–PFPD to analyze the overall sulfur profile of Cheddar cheese.

2. Experimental

2.1. Chemicals

Pure standards were obtained for proper identification of chromatographic peaks: dimethyl sulfide was purchased from TCI America (Portland, OR, USA); dimethyl disulfide, dimethyl trisulfide, dimethyl sulfoxide, and dimethyl sulfone were all obtained from Aldrich (Milwaukee, WI, USA). Carbon disulfide was obtained from EMD Chemicals Inc. (Gibbstown, NJ, USA) and methional (3-methylthiopropionaldehyde) was purchased from Sigma (St Louis, MO, USA). Ethyl methyl sulfide (TCI-EP, Tokyo, Japan) was used as an internal standard for the analysis of VSCs in cheeses with various aging levels (Section 2.4.3).

Gaseous methanethiol was purchased in a cylinder from Aldrich (St Louis, MO, USA) and a solution was prepared by bubbling into methanol. Hydrogen sulfide was prepared by dissolving $Na_2S{\cdot}9H_2O$ (Sigma, St Louis, MO, USA) in acidic water (pH 3). Carbonyl sulfide (COS) was prepared according to the method described in Metrohm Information [27] with some modifications: concentrated sulfuric acid was added dropwise from a dropping funnel to potassium thiocyanate (both from Mallinckrodt/J.T. Baker Inc, Phillipsburg, KY, USA) in a stoppered Erlenmeyer flask; the generated COS (g) was passed through a small diameter glass transfer-line immersed in a cold water bath (for exothermic nature of reaction) and the COS (g) was trapped by bubbling into a separate flask containing distilled water.

2.2. Equipment

2.2.1. GC–PFPD analysis

A Varian CP-3800 gas chromatograph (Varian, Walnut Creek, CA, USA) equipped with a 1077/1079 split/splitless injector port and a pulsed flame photometric detector was used for this study. The volatile compounds extracted by the SPME fiber were thermally desorbed in the 300 °C injector port for 10 min. The injector was in splitless mode for the first four minutes, after which the split valve was opened. Separation of the analytes was performed using a DB-FFAP fused silica capillary column (30 m × 0.32 mm, 1.0 μm film; Agilent, Palo Alto, CA, USA), with nitrogen as the carrier gas (constant flow at 2.0 mL/min). The oven temperature program was as follows: 35 °C held for 5 min, heated to 150 °C at a rate of 10 °C/min, held for 1 min, then heated to 220 °C at a rate of 20 °C/min with a final hold time of 5 min. The PFPD was held at 300 °C and 450 V with the following flow rates: Air1 at 17 mL/min, H_2 at 14 mL/min, and Air2 at 10 mL/min. The detector response signals were integrated using computer software (Star Workstation 6.2, Varian). Statistical analyses were performed with STATGRAPHICS*Plus* software (version 5.0, Manugistics Inc., Rockville, MD, USA).

2.2.2. SPME extraction

A Stableflex 85 μm CAR-PDMS fiber (Supelco, Bellefonte, PA, USA) was used in this study. Prior to use, the fiber was conditioned at 300 °C for 90 min. The fiber was then placed into the SPME adapter of a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) fitted with a vial heater/agitator. For all analyses, agitation during equilibration (10 min prior to fiber exposure) was set to 500 rpm while agitation during extraction was held at 250 rpm.

2.3. Initial sample preparation

Cheddar cheeses with varying ages ("medium", "sharp", and "extra sharp" of two different commercial brands) were purchased from a supermarket and were refrigerated at 3 °C. Samples were used within one month from purchase date. For each cheese sample, a layer of 2 cm was removed from the surface in order to eliminate any possible fluctuations in volatile composition and contamination from packaging. The cheese was then cut into small cubes, measuring approximately 0.5 cm on each side. All vials used in this study were flushed with argon prior to the addition of sample. Following