Chemical Taint in Rindless Gouda Cheese

Owen E. Mills,* Stephen P. Gregory, Frank R. Visser, and Andrew J. Broome

New Zealand Dairy Research Institute, Palmerston North, New Zealand

The compound 2-bromo-4-methylphenol was identified as being responsible for a "chemical (phenolic)" taint in brine-salted Gouda cheese manufactured over a 3 month period. The concentration on the surface of cheese manufactured at the beginning of the period was determined to be between 14 and 25 μg/kg (ppb) decreasing to between 1 and 2 μg/kg at the end. It was proposed that 4-methylphenol and active bromine could be the precursors. Attention focused on the brine, which was shown to be a source of 4-methylphenol and a potential source of bromine. Irradiation of the brine with UV light and dosing with sodium hypochlorite solution were considered to be important factors in generating active bromine. Experiments, carried out in model systems, demonstrated that 2-bromo-4-methylphenol could be generated from sodium bromide and 4-methylphenol in the presence of either UV light or sodium hypochlorite, but not in their absence.

Keywords: *Chemical taint; Gouda cheese; brine salting taint; 2-bromo-4-methylphenol*

INTRODUCTION

A sample of Gouda cheese manufactured at the start of a 3 month production run was observed to have a strong "chemical (phenolic)" taint. Closer inspection of other cheeses manufactured around the same time revealed that the taint was present in cheeses manufactured over about an 8 week period. Furthermore the taint was confined to the surface layer (approximately 1 cm deep) of the 10 kg blocks. An urgent investigation then commenced to identify the taint compound, determine its concentration, and elucidate the mechanism of its occurrence.

Identification of the taint compound and quantitative analysis of the contaminated blocks were carried out in the initial stages of this investigation. Subsequent work was directed at understanding the mechanism of formation of the taint compound. Circumstances pointed to the reaction occurring in the brine tank where the contribution of microflora control measures was considered to be important. The importance of these measures, being the addition of sodium hypochlorite and the passage of brine passed an ultraviolet (UV) light, is discussed in relation to the mechanism proposed for the formation of the taint compound.

MATERIALS AND METHODS

Sensory Evaluation. Sensory evaluation of a range of retention samples covering the period of the taint was carried out systematically. The cheeses were evaluated in reverse order of manufacture by four expert cheese tasters. Samples were cut from the surface (outer 1 cm) of the cheese, and the intensity of the taint was assessed on an hedonic scale of 0−9 where 0 is absent, 1 is threshold, 3 is weak, 5 is moderate, 7 is strong, and 9 is intense. The mean value for the four tasters was calculated for each cheese.

Sample Preparation. The surface layer (outer 1 cm) was cut from a cheese manufactured early in the production run and shredded. A portion (1 kg) was stirred into 3 L of Milli-Q water at 50 °C. The mixture was stirred until the fat had separated from the casein and the casein had formed a cohesive mass. The curd was removed, and excess moisture was returned to the mixture by squeezing the curd. This procedure was repeated twice with fresh batches of shredded cheese using the same water/fat mixture. The final cumulative mixture of fat and water was distilled under vacuum at 60 °C for 2 h. Distillate (800 mL) was condensed in a cold trap at −70 °C. The thawed distillate was continuously liquid/liquid extracted with purified diethyl ether for 6 h. The diethyl ether solution was dried with anhydrous sodium sulfate, then concentrated in a Kuderna Danish apparatus followed by a gentle stream of nitrogen to approximately 100 μL.

Gas Chromatography/Olfactory (GCO) Analysis. Gas chromatography (GC) was carried out using a Shimadzu GC 9A gas chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with a 30 m × 0.25 mm i.d. Econo-Cap capillary column (Alltech Associates Inc.) with either a 25 μm SE-30 phase or a 25 μm Carbowax phase. The column was temperature programmed from 35 to 230 °C at 5 °C/min. Helium carrier gas at 1 kg wt/cm^2 was used.

Olfactory analysis was carried out by sniffing the column effluent which was split equally between a flame ionization detector (FID) and a sniffing port.

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS was carried out using a Shimadzu QP 1000 GC/MS system (Shimadzu Corp., Kyoto, Japan) fitted with either of the same types of columns as described above. The same temperature program as described above was used. Mass spectra were generated at 70 eV. The mass spectrometer was scanned from *m*/*z* 25 to 300 at 1 scan/s.

Extraction and Quantitative Analysis. Steam distillation was carried out in a 5 L, 3-necked round-bottomed flask equipped with a mechanical stirrer and a steam delivery tube and heated by a heating mantle. The distillate was collected in a 1 L Erlenmeyer flask cooled in ice/water. Cheese cubes (approximately 1 cm^3, 500 g) were mixed in reverse osmosis (RO) water (1750 mL). Concentrated sulfuric acid (150 mL) and internal standard solution (1 mL of aqueous solution 2,4-dichlorophenol at 24.6 μg/mL) were also added. Distillate (450 mL collected in 25 ± 2 min) was transferred to a 500 mL separatory funnel and extracted with 5 × 25 mL of pentane. The combined pentane extracts were washed once with 5 mL of saturated NaCl solution, passed through a glass wool plug into a 250 mL Erlenmeyer flask, and dried for at least 1 h on anhydrous $MgSO_4$ (heated for 5 h at 260 °C). The anhydrous solution was passed through a Whatman No. 2 fluted filter into a Kuderna Danish apparatus and evaporated to approximately 2 mL. More solvent was removed by a gentle stream of nitrogen to a final volume of approximately 0.1 mL. A 1 μL aliquot of this concentrate was analyzed by GC/MS in the single ion monitoring (SIM) mode using a 30 m capillary column with a Carbowax phase. Conditions used for the analysis are described above.

Quantitation was carried out using the sum of the areas of *m*/*z* 186 and 188 for 2-bromo-4-methylphenol and *m*/*z* 162, 164,

* To whom correspondence should be addressed.

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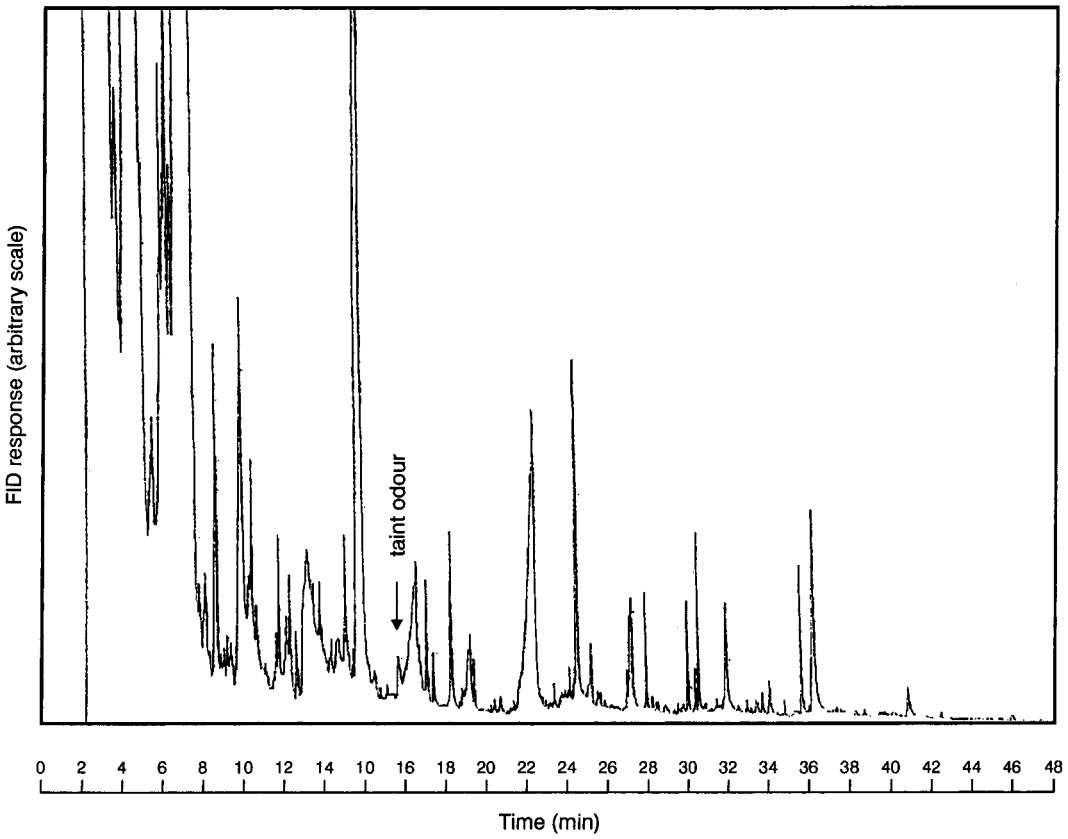

Figure 1. Volatiles from tainted cheese chromatographed on a capillary column with an SE-30 phase.

and 166 for 2,4-dichlorophenol. The response factor, relative to 2,4-dichlorophenol, was calculated as the sum of the areas of the 2-bromo-4-methylphenol peaks divided by the sum of the areas of the 2,4-dichlorophenol peaks, adjusted for equal amounts injected. The recovery of 2-bromo-4-methylphenol, relative to the recovery of 2,4-dichlorophenol, was determined by analyzing noncontaminated Gouda cheese, spiked with known amounts of 2-bromo-4-methylphenol and 2,4-dichlorophenol, and comparing the determined peak ratio with the expected ratio. Finally, the concentration of 2-bromo-4-methylphenol in the surface layer of the cheese was calculated.

Accuracy and Precision. Because of the time constraint on finding a solution to this problem, very little replication was done. The quantity measured most frequently was the response factor for 2-bromo-4-methylphenol, which was determined 10 times over a 15 day period during which assays were carried out. The duplicate analysis of one cheese sample only (day 16) gave an indication of the reproducibility of the method. Two other samples that were also analyzed in duplicate (days 47 and 57) showed such low values (close to or at the detection limit) that the figures obtained from them could not be used to draw conclusions about reproducibility. All other samples were analyzed only once. The result of all this is that the final figures can only be given as a rather large range with a confidence of 95%.

Experiments with Brine. Samples of brine were taken for the following experiments immediately before and after the UV light source. 5-Sulfosalicylic acid (40 g) was stirred into brine (1 L) for 10 min. The resulting mixture containing coagulated protein was centrifuged at 6500*g* for 10 min. The supernatant was decanted and liquid/liquid extracted with diethyl ether for 7 h. The diethyl ether solution was dried and concentrated as described above.

Phenol (0.1 g) and 4-methylphenol (0.1 g) were dissolved in 1 L of brine taken before passage through the ultraviolet light source. The solution was placed in a 1 L beaker of 10 cm diameter and irradiated from directly above with a bank of four UV light tubes (mercury vapor fluorescent tubes, each 15 W, 253.7 nm). The solution was stirred, and the whole assembly was placed at 10 °C for 89 h. A similar experiment was set up for brine taken after passage through the UV light source except that the solution was held in the dark for 89 h at 10 °C. At the end of these experiments, the brine solutions were treated as described above to obtain a solution for GC/MS analysis.

Model Systems. Sodium bromide (100 g) was dissolved in 2 L of Milli-Q water and adjusted to approximately pH 4.7 with 0.1 M HCl. Sodium thiosulfate pentahydrate (0.78 g) was added to reduce residual bromine to bromide at the start of the experiment. The solution was stirred for 30 min. Phenol (0.2 g) and 4-methylphenol (0.2 g) were then added, and the pH was readjusted to 4.7. This solution was divided into 2 × 1 L volumes. One 1 L volume was further divided into 2 × 500 mL volumes. One 500 mL volume was stirred in the presence of UV light and one in the dark at 10 °C for 72 h. Sodium hypochlorite solution (10 mL, 12% available chlorine) was added to the second 1 L volume which was then divided into 2 × 500 mL volumes. One 500 mL volume was stirred in the presence of ultraviolet light and one in the dark at 10 °C for 72 h. After 72 h, each of the four solutions was liquid/liquid extracted against diethyl ether for 6 h and samples were prepared for GC/MS analysis as described above.

RESULTS AND DISCUSSION

Identification and Quantitation of the Taint Compound. The retention time, on a column with an SE-30 phase, of a compound having an odor characteristic of the taint is shown in Figure 1. Although the chromatography was better on a Carbowax column, illustrated by sharper peaks for free fatty acids, the unknown odor compound coeluted on the Carbowax column with a major peak which was identified as octanoic acid. On the SE-30 column, the unknown odor compound eluted as a peak which was reasonably well resolved from other peaks (Figure 1).

The sample containing cheese volatiles was then analyzed by GC/MS using an SE-30 column. The mass

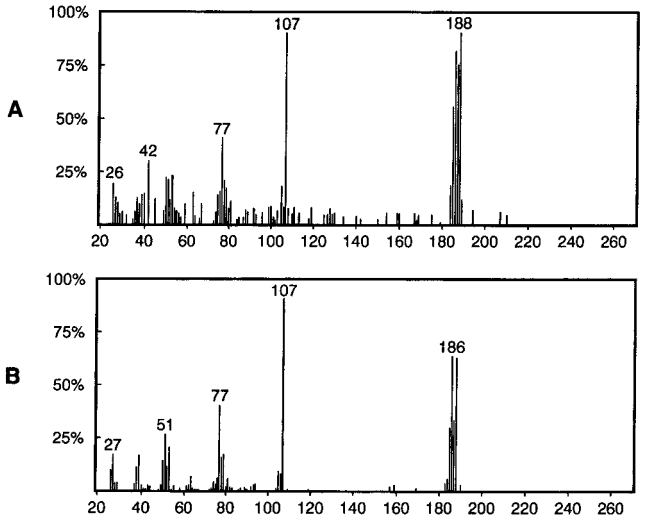

Figure 2. Mass spectrum of (A) unknown taint compound and (B) 2-bromo-4-methylphenol.

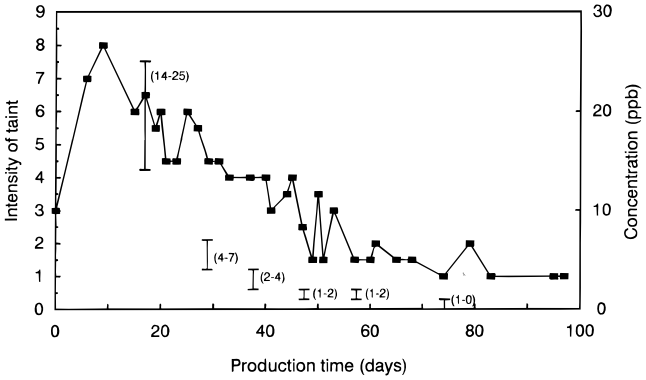

Figure 3. Mean taint intensity (four tasters) versus production time indicated as days from the beginning of the production run. Intensity was assessed on a scale of 0 to 9 where 0 is absent, 1 is threshold, 3 is weak, 5 is moderate, 7 is strong, and 9 is intense. Also shown is the concentration range for 2-bromo-4-methylphenol for selected samples.

spectrum of the scan at the top of the peak corresponding to the unknown odor compound is shown in Figure 2A. A tentative identification was obtained by comparing this spectrum with those in the *Wiley Registry of Mass Spectral Data* (Wiley, 1994). Authentic 2-bromo-4-methylphenol was obtained (Aldrich Chemical Co.) and shown to have a mass spectrum very similar to (Figure 2) and retention time identical to that of the unknown. A volatile extract prepared from an untainted cheese did not show any evidence of this compound. All tasters confirmed that cheese spiked with 2-bromo-4-methylphenol had the same flavor as the complaint cheese.

Initial experiments with direct extraction of cheese were unsuccessful in rapidly providing a sample for quantitative analysis. The stability, limited volatility, and moderate solubility in water of 2-bromo-4-methylphenol suggested the use of steam distillation at atmospheric pressure. The clean fat-free and protein-free distillate could then be extracted with a suitable solvent and evaporated to a concentrate that could be analyzed by GC and GC/MS/SIM. Early tests showed that steam distillation required a large amount of acid to break down the cheese completely, thus liberating the analyte and eliminating problems of foam formation. Pentane was chosen as the solvent for extracting the distillate because of its total immiscibility with water and its low boiling point. It was assumed that the solubility of 2-bromo-4-methylphenol in pentane was high enough to isolate sufficient material to allow quantitation (using 2,4-dichlorophenol as an internal standard). Mass spectrometric analysis had shown that the molecular ion peaks were not contaminated by significant fragment peaks of other compounds coeluting with 2-bromo-4-methylphenol, and they could be used for quantitation by SIM.

Figure 3 shows that the taint was perceived strongly in the early days of production and decreased in intensity thereafter. After about 35 days, the taint was only weakly perceived, and from about 55 days, the taint was only perceived at threshold levels. The surface of brine-salted cheese, unlike the interior, typically has a "stale", "slightly oxidized", "slightly chemical (chlorine)" flavor due to direct contact with the brine. At an intensity of 3 or less, the taint was considered to be present at a level similar to this background flavor and thus the flavor profile on the surface of the cheese was considered to be "normal". For several of the retention samples, a plug sample was also taken and the central portion of the cheese was evaluated. There was nothing atypical about the flavor profile of any of the plug samples evaluated.

Time did not allow optimization of the quantitation procedure. In addition a coefficient of variation of 14% was obtained for the relative responses of 2-bromo-4-methylphenol and 2,4-dichlorophenol by GC/MS/SIM from 10 repeated injections of a mixture of the two compounds. Therefore, the data obtained from analysis of a selected number of samples shown in Figure 3 are presented as concentration ranges. The data show that there was a significant decrease in concentration during the first 38 days. This trend is in agreement with flavor intensity versus time of manufacture.

A Proposed Mechanism for the Formation of the Taint Compound. The Gouda cheese making process, the subject of this investigation, is a fully automated one. Curd particles are formed in an enclosed vat, then pumped into a mould in which the final 20 kg block is formed. Whey is drained from the bottom, and the blocks are then conveyed to the brine tank. Because the taint was only present on the surface of the blocks, the problem must have occurred after block formation, although precursors of the taint compound may have entered the process prior to this point. It was proposed that 4-methylphenol and active bromine could be the precursors of the taint compound. 4-Methylphenol occurs naturally in New Zealand cow's milk (Keen et al., 1994) and can also be produced by cheese-related microorganisms (Guthrie, 1994). Hup et al. (1982) implicated lactobacilli and "weak" brine (<14% w/v NaCl) in the production of a "phenolic" flavor defect in rinded Gouda cheese, although they did not identify the compound(s) responsible for the defect. Bromine is a minor contaminant of cheese salt, although it is present in a very stable form as sodium bromide. Attention logically focused on the brine as the source of the problem because it conceivably could contain both 4-methylphenol, expelled with the cheese whey or maybe produced by fermentation, and bromine in salt.

When 2-bromo-4-methylphenol was identified as the major odor compound responsible for the taint, a search was carried out in the total ion current of the GC/MS chromatogram for 2-bromophenol. Justification for this search was based on the knowledge that phenol and 4-methylphenol, together with several other phenol

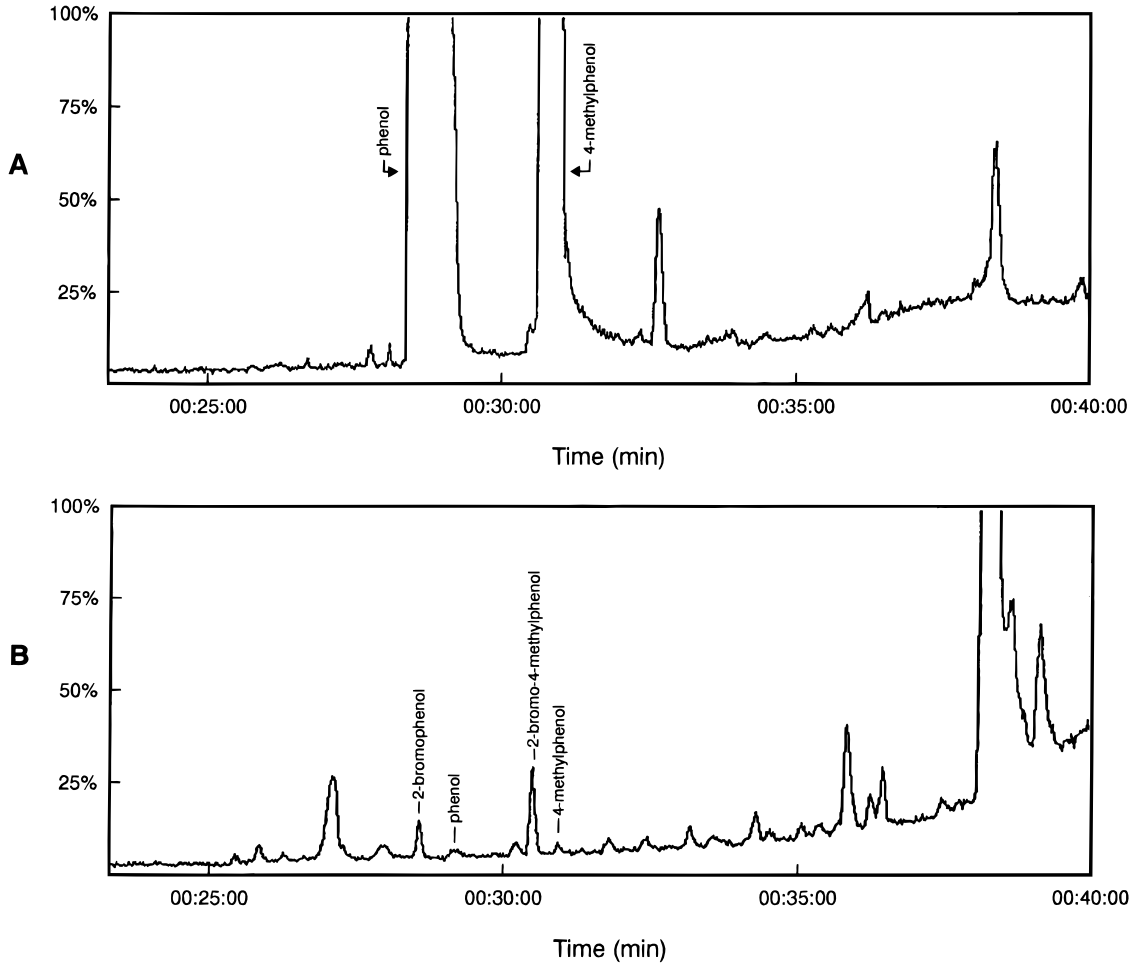

Figure 4. Total ion current of reaction products of model system studies chromatographed on a capillary column with a Carbowax phase. The study was carried out in the dark in (A) the absence and (B) presence of sodium hypochlorite.

derivatives, are found to occur naturally in New Zealand milk (Keen et al., 1994) and if this pool of phenolic compounds is the source of the phenolic precursor for 2-bromo-4-methylphenol, then it could also be the source of other phenolic precursors. Phenol and 4-methylphenol are the most abundant phenolic compounds in milk. Although bromophenol was found, it was not detected during GCO analysis.

A mechanism was proposed for the formation of 2-bromo-4-methylphenol and 2-bromophenol in the brine from the reaction of bromine with 4-methylphenol and phenol, respectively. Although 2-bromo-4-methylphenol and 2-bromophenol were not detected in the brine, they may have been present below detection limits. However, detection of these compounds in the cheese probably results from a concentration effect at the cheese surface. Both phenol and 4-methylphenol were positively identified in the brine from their full-scan mass spectra and retention times. A likely source of bromine was thought to be the salt which was manufactured from sea water. Salt periodically added to the brine tank to maintain the concentration, on analysis, was found to have a concentration of 45 ppm sodium bromide. The water used to make up the brine was shown to be an insignificant source of bromide compared to the salt.

If the reaction, described above, for the formation of 2-bromo-4-methylphenol and 2-bromophenol is accepted, then a mechanism is required to explain the conversion of bromide to a reactive bromine species. This could occur either by oxidation promoted by hypochlorite in the brine or by the generation of bromine radicals by UV light. Treatment of the brine with hypochlorite and UV light is carried out to suppress yeasts and moulds. This action is necessary as the brine tank is so large that regular renewal of the brine is impractical. Model experiments were therefore designed to establish the importance of hypochlorite addition and UV treatment to the proposed mechanism for the formation of 2-bromo-4-methylphenol and 2-bromophenol.

In the experiments in which 4-methylphenol and phenol were added to brine prior to UV irradiation, 2-bromo-4-methylphenol was positively identified from its full-scan mass spectrum and retention time. 2-Bromophenol was tentatively identified from its mass spectrum. These results confirmed that the reaction between the phenolic compounds and active bromine was possible in the brine. The next stage was to determine what factors were necessary to liberate bromine. As the brine already contained sodium hypochlorite, the importance of that factor, independent of other factors, could not be determined; therefore, the use of a model system was advocated. The choice of sodium bromide instead of sea salt was made to enhance the effect. The results of conducting these studies in the dark and in the presence or absence of hypochlorite are shown in Figure 4. The two large peaks in Figure 4A (no hypochlorite) are sufficiently large that it would appear that if any bromophenols were present they would be hidden by the precursor peaks. However, their

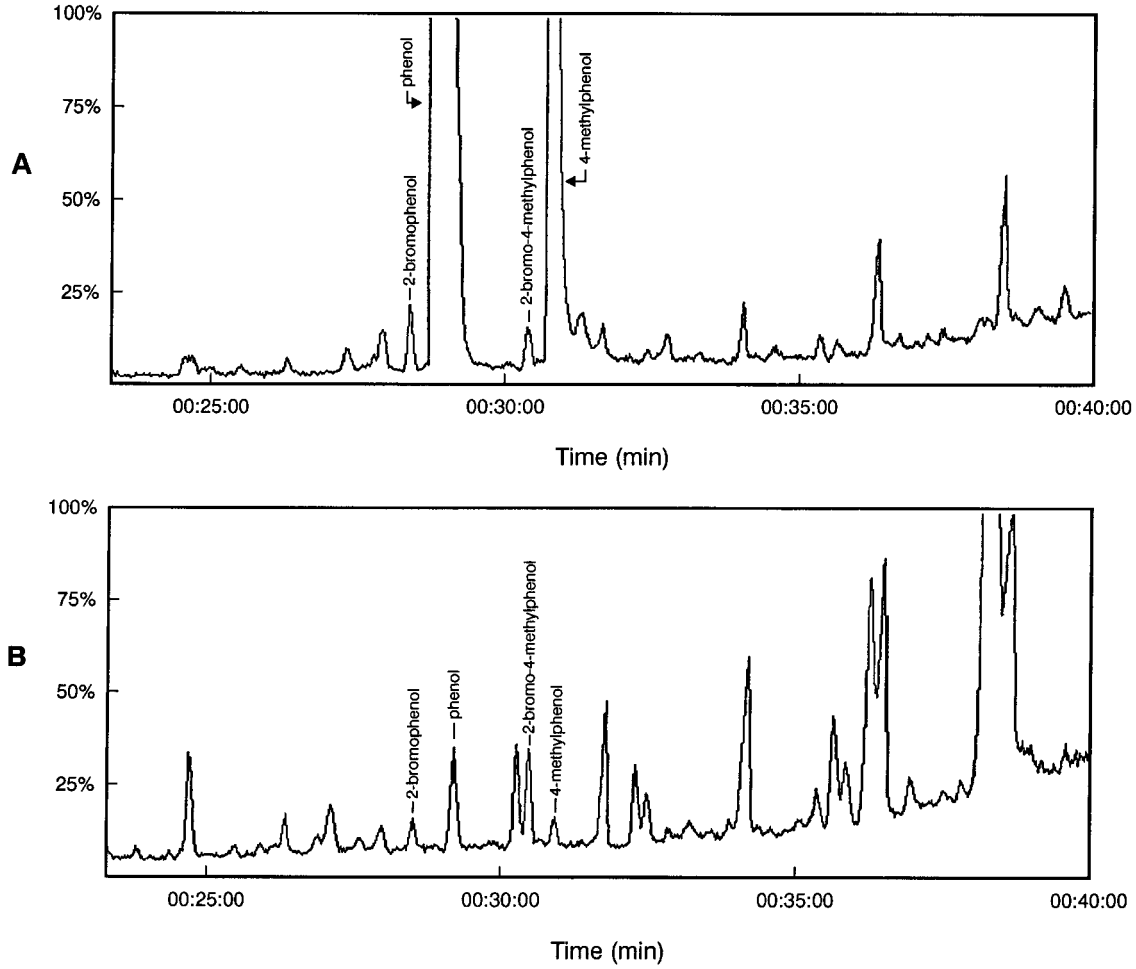

Figure 5. Total ion current of reaction products of model system studies chromatographed on a capillary column with a Carbowax phase. The study was carried out in the presence of ultraviolet light and (A) in the absence and (B) presence of sodium hypochlorite.

absence was indicated by the lack of 172/174 and 186/188 ion pairs at their respective retention times. In the presence of hypochlorite, extensive reaction occurred and both 2-bromophenol and 2-bromo-4-methylphenol were generated (Figure 4B). The results of conducting these studies in the presence of UV light and in the presence or absence of hypochlorite are also shown in Figure 5. 2-Bromophenol and 2-bromo-4-methylphenol were generated under both sets of conditions. Although it was found that bromophenols were formed in the presence of either UV light or hypochlorite, the relative importance of these two factors cannot be determined from these experiments.

The most likely source of phenols in the brine was cheese itself. At the beginning of the manufacturing run, the brine would have already contained phenols derived from the previous use of the brine. Between manufacturing runs, when no cheese was present in the brine, the brine was continuously treated by hypochlorite addition and UV irradiation. This could have resulted in the formation and accumulation of the bromophenol compounds in the brine. When blocks of cheese were introduced to the brine at the beginning of the new run, some of the bromophenols would have been adsorbed onto the surface of the cheese. Provided the rate of removal of the taint compound was higher than the rate of formation, the concentration of the compound in the brine would reduce. This would explain the high levels of the taint followed by a steady decrease as the run progressed (Figure 3). Alternatively bromophenol compounds could be formed in the cheese surface layer as bromine is soluble in fat. This hypothesis also fits the data in Figure 3 except that in this case active bromine will have accumulated in the brine between manufacturing runs rather than the bromophenol compounds and the level would decrease due to absorption in the cheese. The rate of absorption would have to be greater than the rate of formation of active bromine.

Active bromine, required to form bromophenol compounds, can be generated from bromide ions by the action of hypchlorite. In water, the salt hydrolyses to hypochlorous acid, which forms chlorine in an equilibrium reaction. The chlorine oxidizes the bromide to free bromine (Cotton and Wilkinson, 1988). Bromination of 4-methylphenol is directed to the favored 2-position (Fieser and Fieser, 1956). The formation of similar chloro compounds does not take place to any measurable extent because hypochlorous acid is primarily an oxidizing agent in itself and does not liberate chlorine from sodium chloride. There are reports in the literature implicating bromophenols in the flavor of potable water supplies (Sithole and Williams, 1986), the marine environment (Boyle et al., 1993), and some food products (Steeg et al., 1990). However, in all these instances, the compound brominated is phenol whereas the work reported here describes a brominated cresol.

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