

### Abstract

Primary and secondary amines were isolated from whey as their 2,4-dinitrophenyl derivatives, separated by thin-layer partition chromatography, and identified by gas chromatography-mass spectrometry. Examination of raw and pasteurized sweet whey, reconstituted spray dried whey powder, and protein-free ultrafiltration permeate of sweet and acid wheys revealed the same qualitative distribution of amines. Amines identified were methyl-, ethyl-, isobutyl-, dimethyl-, isopropyl-, and n-amylamine, pyrrolidine, and piperidine.

### Introduction

Earlier studies of amines in milk were primarily concerned with their contribution to flavor and aroma. Milk has been reported to contain n-propyl- and n-hexylamine (2) and methyl-, ethyl-, butyl-, and dimethylamine and possibly pyrrolidine (8). All compounds were only tentatively identified either by retention time (2) or relative mobility (8).

We recently investigated the primary and secondary amines in whey using mass spectrometry for positive identification. The present concern regarding the role of secondary amines in the formation of nitrosamines together with the possibility of identifying naturally occurring pro- and antioxidants prompted the investigation.

### Materials and Methods

Whey samples were prepared in this laboratory. The following were investigated: (a) raw, sweet whey from Cheddar cheese manufacture, (b) the same whey after pasteurization, (c) reconstituted (50 g/liter) spray-dried sweet whey, (d) a protein-free permeate obtained by ultrafiltration (3) of Cheddar cheese sweet whey containing 5% solids, (e)

a protein-free permeate obtained by ultrafiltration of cottage cheese acid whey containing 5% solids, and (f) a concentrate of cottage cheese whey containing 35% solids.

*Formation of 2,4-dinitrophenyl derivatives (DNPs).* Whey (250 ml) was adjusted to pH 8.0 to 8.5 by the addition of solid potassium carbonate. The precipitate which formed was removed by centrifugation. Acetone (10 ml), and .5 ml 1-fluoro-2,4-dinitrobenzene (FDNB) were added to the supernatants, and the mixture was stirred magnetically at room temperature. After 18 h it was extracted with four 100-ml portions of dichloromethane with the emulsion broken each time by centrifugation. The combined extracts were evaporated to dryness under a stream of nitrogen. The procedure was repeated exactly on whey samples adjusted to pH 11.0 to 11.5 with solid potassium carbonate.

The DNP's were streaked across a 20.3 × 25.4 cm thin-layer partition plate (6) and developed with hexane:benzene (65:35) (6). The colored bands were scraped from the plate and the scrapings transferred to the top of a bed of 8% hydrated alumina (5) contained in a chromatographic tube (1 cm od × 12 cm) filled dry to a height of 1.5 to 2.0 cm. The colored band was eluted with hexane:benzene (1:1) or, when necessary, with benzene. When straight benzene was used, relatively large amounts of stationary phase (polyethylene glycol 400) eluted with the colored band. The polyethylene glycol 400 was removed by evaporating the benzene effluent to about 1 ml, adding hexane to cause a visible turbidity (4 to 5 ml), and extracting with three 4-ml portions of water. The organic layer was evaporated to dryness.

All bands were rechromatographed on small alumina columns just before analysis in the mass spectrometer. The DNP dissolved in 10% dichloromethane in hexane was transferred to a Pasteur pipet (145 mm × 7 mm) containing a bed of 8% hydrated alumina 1.5 to 2.0 cm in height. The column was washed with 3 column volumes of 10% dichloromethane in hexane, and the effluents were discarded. The column was then eluted with dichloromethane,

Received April 19, 1974.

<sup>1</sup> Research conducted while Dairy Products Laboratory was located in Washington, D.C.

collecting only the colored effluent.

*Gas-liquid chromatography (GLC)-mass spectrometry (MS)*. Each DNP (about 5  $\mu$ g) was analyzed by GLC-MS. A 122  $\times$  .16 cm silanized stainless steel column packed with 3% OV-225 on 80/100 mesh Gas Chrom Q was used in the LKB 9000 mass spectrometer. The column was operated isothermally at 200 C. Other conditions were: flash heater and separator, 230 C; ion source, 290 C. Chromatographic peaks were scanned repeatedly near the apex over a m/e range of 12 to 450. Scanning time was 4.5 s at an accelerating voltage of 3500 V with an electron energy of 70 ev.

DNP standards were prepared following tentative identification of the unknowns (1), and their mass spectra, retention time, and relative mobility compared to those of the unknown. Mass spectra of DNP amines have been reported (9).

### Results and Discussion

Figure 1 is a typical thin-layer partition chromatogram from which the following DNP amines were positively identified: methyl-, ethyl-, isopropyl-, isobutyl-, n-amyl-, and dimethylamine, pyrrolidine, and piperidine. These were identified in all samples of whey

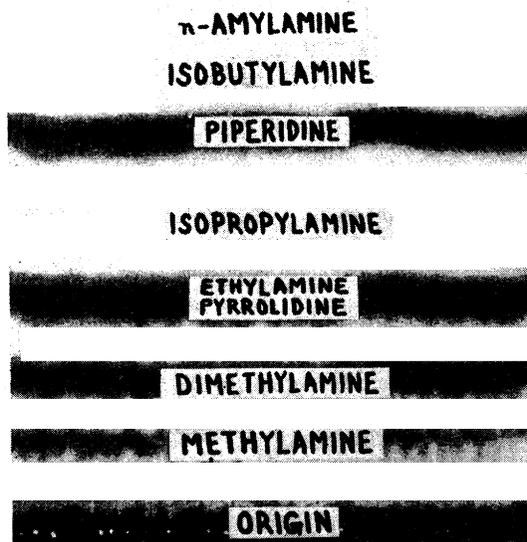


FIG. 1. Separation of amines isolated from whey as their 2,4-dinitrophenyl derivatives. The isopropyl-, isobutyl-, and n-amylamine bands are not visible on the photograph. Band at origin contains 2,4-dinitroaniline and 2,4-dinitrophenol but was not analyzed further. Stationary phase, polyethylene glycol 400; mobile phase, n-hexane; benzene (65:35) saturated with stationary phase.

although relative amounts varied. Ethylamine, dimethylamine, and piperidine occurred in the highest concentration; methylamine and pyrrolidine were in lesser amounts; and isobutylamine was in significantly smaller amounts. Only traces of isopropylamine and n-amylamine were detected. From the density of the bands, it is estimated roughly that the wheys contained less than 1 mg/liter of the total number of amines identified.

Variations in the procedure were introduced to check whether some or all of the amines were artifacts. These included (a) boiling sweet whey, cooling to room temperature and adjustment of the pH to 11.0 to 11.5; (b) adjusting the pH of sweet whey to 11.0 to 11.5 followed by boiling; and (c) reacting sweet whey at its normal pH (about 6.0) with FDNB for 40 h. Both (a) and (b) gave normal patterns of DNP amines which were quantitatively similar to the whey samples treated in the normal manner. Variation (c) also gave the normal pattern, but in this case overall yield of the DNP amines was lower. A band subsequently identified as O-DNP ethanol was noted. Ethanol was not found in any of the other DNP experiments, but it was detected along with the other amines when an extract of whey was reacted with pyruvic acid chloride 2,6-dinitrophenylhydrazine (5).

The pattern of amines in human blood (3) is similar to that in the whey samples.

### Acknowledgment

Frank McDonough, Dairy Foods & Nutrition Laboratory of the Nutrition Institute, Agricultural Research Center, Beltsville, Maryland, supplied the whey samples.

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