

Evidence for Methional in Skim Milk Exposed to Sunlight

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ABSTRACT

Methional in milk exposed to sunlight has been questioned by several researchers (9, 14) since Patton and Josephson (4, 5) suggested its resemblance to the light activated flavor in skim milk. This report establishes the presence of methional in skim milk exposed to direct sunlight and summarizes our observations.

EXPERIMENTAL PROCEDURE

One liter of commercial, pasteurized skim milk was exposed to direct sunlight in 4 cm by 60 cm glass chromatographic columns until a definite activated flavor developed (approximately 40 to 50 min). The exposed sample was subjected to negative pressure dialysis (7) employing four 1-liter vacuum flasks at 2.2 C for 18 h. Seven hundred milliliters of sera were extracted successively with 40 ml and 20 ml of methylene chloride in a 1-liter separatory funnel, and the combined methylene chloride layers centrifuged at 2000 rpm for 10 min to break the slight emulsion. The recovered methylene chloride was dried with sodium sulfate and passed through a 2,4-dinitrophenylhydrazine (DNP hereafter refers to dinotrophenyl) reaction column (11). The 2,4-DNPhydrazones were separated from the 2,4-DNPhydrazine stripped from the reaction column by the methylene chloride on Dowex 50² (12). The ion-exchange column effluent containing the 2,4-DNPhydrazones was evaporated to dryness. The 2,4-DNPhydrazones were taken up in 5 ml of chloroform and separated into carbonyl classes according to (13). Following elution of the large ketone 2,4-DNPhydrazone band, the column was eluted with 75 ml of a 25% methanol in chloroform solution. The 25%

methanol-chloroform eluant was evaporated to dryness and the 2,4-DNPhydrazone residue dissolved in .3 ml of methylene chloride. The 2,4-DNPhydrazone solution was spotted across .5 mm thick precoated preparative Silica Gel G thin layer chromatographic plates (Analtech, Inc., Newark, DE), and the plates were developed in benzene. The area corresponding to the R_f value of authentic methional 2,4-DNPhydrazone was scraped from the air dried plates and the 2,4-DNPhydrazones eluted from the absorbent with methylene chloride.

GAS LIQUID CHROMATOGRAPHY (GLC) AND MASS SPECTROMETRY (MS)

Carbonyl compounds were regenerated from the isolated 2,4-DNPhydrazone fraction for GLC-MS studies by the injection system of Wong and Schwartz (15). The 2,4-DNPhydrazone fraction was absorbed from hexane-benzene (1:1) solutions on silicic acid packed capillaries, 10 μ l of 8% aq. phosphoric acid were added, and the carbonyls were regenerated in the flash heater of the LKB 9000 Combination Gas Chromatograph-Mass Spectrometer.

Gas liquid chromatography was at 70 C in a 3.04-M by .32-cm stainless steel column packed with 7.5% ethylene glycol adipate and 2% phosphoric acid on Anakrom ABS (Analabs, Inc., Hamden, CT). Helium served as the carrier gas. The temperatures of the flash heater and molecular separator were maintained at 200 C. Mass spectra were obtained by repeated scanning at the previously determined GLC elution time of authentic methional at a constant accelerating voltage of 3.5 kv, an electron energy of 70 ev, and a scanning time of 2 s over a m/e range of 15 to 150.

RESULTS AND DISCUSSION

As previously reported by Samuelsson and Thome (10), an intense activated flavor developed in cultured buttermilk exposed to direct sunlight. Furthermore, the sera obtained by negative pressure dialysis also developed the intense flavor on exposure indicating the flavor compound(s) originated from a nonprotein

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source and presumably free amino acids as suggested by Samuelsson and Thome (10). The presence of relatively substantial quantities of methional in a methylene chloride extract of 700 ml of buttermilk sera (exposed both before and after dialysis) was confirmed by GLC-MS following extraction of acidic compounds with 1 N NaOH and evaporation to .2 ml.

Initial attempts to identify methional in exposed skim milk by this procedure were unsuccessful despite the organoleptic detection of methional in the methylene chloride extract. The concentration in exposed skim milk is substantially less than in exposed buttermilk. In addition, losses incurred during negative pressure dialysis and evaporation of the methylene chloride extract of the serum to a volume suitable for GLC-MS analysis resulted in levels below the limit of detection by the mass spectrometer. Conversion of the suspected compound to its 2,4-DNPhydrazone followed by isolation as outlined in the experimental procedure not only reduced losses as a result of concentrating the methylene chloride extract but provided a means for injecting the entire isolated sample onto the GLC column for mass spectral analysis. Preliminary studies on the derivitized flavor compound suggested, however, a loss in concentration during prolonged handling and storage. Therefore, mass spectral analyses on the regenerated compound were accomplished within 24 h of derivitization to limit such losses.

The mass spectrum of the regenerated methional isolated from 700 ml of sera of exposed skim milk, although weak, revealed the characteristic fragment pattern of authentic methional. Discrepancies in the relative intensities of several fragments occurred as a result of errors inherent in weak spectra in the presence of considerable background noise resulting from the required multiplier voltage necessary for high sensitivity.

Confirming evidence for methional in sunlight-exposed skim milk was obtained by exposing 700 ml of skim milk sera to sunlight for 10 to 15 min. The increased concentration of methional isolated as a result of eliminating losses incurred during negative pressure dialysis resulted in a mass spectrum of adequate intensity. A comparison of the relative intensities of the characteristic fragments of authentic methional and methional isolated from sunlight-ex-

TABLE 1. Mass spectra of authentic methional and methional isolated from skim milk serum exposed to sunlight.

m/e	Relative intensity	
	Authentic	Isolated
48	100.0	100.0
47	44.2	58.2
104	33.2	32.1
76	27.6	22.7
45	25.2	24.5
61	18.6	25.1
55	12.6	21.2
106	3.7	2.5

posed milk serum are in Table 1.

Skim milk contains 1.3 to 4.3 μM /liter free methionine (1, 3, 10), which represents a potential methional concentration three to nine times greater than its reported 50 ppb flavor threshold in skim milk (4). Determining the significance of methional in the activated flavor is complicated by the report of Ballance (2) that, although methional is the initial product of the Strecker degradation of methionine, continued heating in the presence of ninhydrin results in a preponderance of methyl mercaptan with traces of acrolein, isobutyraldehyde, dimethylsulfide, and dimethyldisulfide in the reaction mixture. Indeed, Samuelsson (8, 9) has concluded that methyl mercaptan, and to a lesser degree sulfides, disulfides, and hydrogen sulfide arising from both free and peptide-linked methionine are responsible for the activated flavor in skim milk.

Our own organoleptic evaluations of skim milk and an aqueous solution of methionine and riboflavin exposed to sunlight led us to conclude that methional is initially the dominant flavor compound while methyl mercaptan arises on continued exposure. Skim milk and aqueous solutions of methionine (2.7 μM /liter) and riboflavin (4.8 μM /liter) were exposed to direct sunlight in 1.5 cm by 15 cm test tubes for varying times. Within 10 min, the methionine-riboflavin solution had a definite methional-like character which changed to an extreme methyl mercaptan-like odor and flavor following an additional 10 min of exposure. The flavors were less pronounced in skim milk and required an additional 5 min for development, but flavor changes were the same. It

appears, therefore, that not only is the degree of the off flavor dependent on the intensity of the light source and length of exposure (6), but also on the compounds responsible for the off-flavor.

REFERENCES

- 1 Armstrong, M. D., and K. N. Yates. 1963. Free amino acids in milk. *Soc. Exp. Biol. Med.* 113:680.
- 2 Ballance, P. E. 1961. Production of volatile compounds related to the flavor of foods from the Strecker Degradation of DL-methionine. *J. Sci. Food Agr.* 12:532.
- 3 Hendrickx, H., H. De Moor, and A. Huyghebaert. 1955. Free amino acids in freshly drawn whole milk. *Neth. Milk Dairy J.* 20(2):123.
- 4 Patton, S. 1954. The mechanism of sunlight flavor formation in milk with special reference to methionine and riboflavin. *J. Dairy Sci.* 37:446.
- 5 Patton, S., and D. V. Josephson. 1953. Methionine—origin of sunlight flavor in milk. *Science* 118:211.
- 6 Parks, O. W. 1974. Page 257 in *Fundamentals of dairy chemistry*. B. H. Webb, A. H. Johnson, and J. A. Alford, eds. Avi Publishing Co., Westport, CT.
- 7 Parks, O. W., D. P. Schwartz, K. Nelson, and C. Allen. 1967. Evidence for kynurenine in milk. *J. Dairy Sci.* 50:10.
- 8 Samuelsson, E. G. 1962. Model experiments on sunlight flavour in milk - di - and tripeptides of methionine. Page 552 in *Proceedings 16th Int. Dairy Congress, Copenhagen*. Vol. A, Section 11:1.
- 9 Samuelsson, E. G. 1962. Experiments on sunlight flavour in milk-S³⁵ labeled milk. *Milchwissenschaft* 17:401.
- 10 Samuelsson, E. G., and K. E. Thome. 1959. Investigations on the influence of light on milk as a contribution to the knowledge on sunlight flavor. Page 488 in *Proceedings 15th Int. Dairy Congress, London*. Section 2B.
- 11 Schwartz, D. P., H. S. Haller, and M. Keeney. 1963. Direct quantitative isolation of monocarbonyl compounds from fats and oils. *Anal. Chem.* 35:2191.
- 12 Schwartz, D. P., A. R. Johnson, and O. W. Parks. 1962. Use of ion exchange resins in the micro analysis of 2,4-dinitrophenylhydrazones. *Microchem. J.* 6:37.
- 13 Schwartz, D. P., O. W. Parks, and M. Keeney. 1962. Separation of 2,4-dinitrophenylhydrazone derivatives of aliphatic monocarbonyls into classes on magnesia. *Anal. Chem.* 34:669.
- 14 Wishner, L. A., and M. Keeney. 1963. Carbonyl pattern of sunlight-exposed milk. *J. Dairy Sci.* 46:785.
- 15 Wong, N. P., and D. P. Schwartz. 1969. A versatile injector for the gas chromatograph. *J. Chromatogr. Sci.* 7:569.