Degradation of ^{35}S-Methionine to Methyl Mercaptan in Surface-Ripened Cheese

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^{35}S-Methionine was injected into wheels of Trappist-type cheese both with and without the normal growth of surface organisms. After three days of incubation, the volatile sulfur compounds of the cheeses were separated into hydrogen sulfide, mercaptans, and organic sulfides and disulfides by means of a differential trapping system. By liquid scintillation and thin-layer chromatography, it was possible to show methionine as the precursor of methyl mercaptan in the cheese having surface growth.

Methyl mercaptan (CH_3SH) has been detected in soft surface-ripened cheese (Jarczynski and Kiermeier, 1956), semisoft white mold cheese (Tsugo and Matsuoka, 1962), and Cheddar cheese (Libbey and Day, 1963), but its flavor significance in these cheeses was not clearly established. Recently, the authors reported the identification of CH_3SH and hydrogen sulfide in surface-ripened cheese of the Trappist type and presented evidence showing CH_3SH as the primary compound contributing to the strong, putrid aroma normally associated with this cheese (Grill *et al.*, 1966).

The mechanism of flavor development, more specifically the mechanism of CH_3SH development in surface-ripened cheese of the Trappist or Limburger type, has not been elucidated. Generally, it is believed that the strong aroma which develops is principally the result of metabolism by microorganisms, particularly *Brevibacterium linens*, present on the cheese surface (Foster *et al.*, 1957). This opinion arises from the fact that the putrid aroma becomes noticeable with the appearance of *B. linens* and also from the fact that pure cultures of *B. linens* have such odor. In the case of white mold cheese, Tsugo and Matsuoka (1962) have shown that an enzyme preparation of *Penicillium caseicolum* is capable of splitting free methionine to CH_3SH, ammonia, and α-ketobutyric acid.

Thus, with methionine being one of the major sources of sulfur in Trappist-type cheese, it was important to determine if this amino acid was the precursor for CH_3SH, and whether surface organisms found on Trappist cheese were necessary for degradation of methionine into volatile sulfur compounds.

Experimental

Preparation of Sample. From a commercial lot of green Trappist cheese (manufactured at the university creamery), several wheels were brined in freshly prepared brine solution, waxed, and cured for 21 days. The remaining wheels of cheese were brined, cured, and waxed after 21 days of ripening under normal production conditions. By using this procedure, surface growth was prevented in the former group of cheeses, while the latter group developed the usual growth of surface organisms and their enzyme systems. Several members of the Dairy Department staff evaluated the flavor and texture characteristics of samples from each cheese group after 24 days of ripening. After the cheese had been evaluated, ^{35}S-methionine (70.41 μc., specific activity 20 mc. per mm., dissolved in 5 ml. of distilled water) was injected into a wheel of cheese from each group and the two cheeses were incubated for 3 days at 7° C. The injections were localized to a 100-gram section in each wheel.

Determination of Radioactivity in Volatile Sulfur Compounds. The apparatus used for the removal of the volatile sulfur compounds consisted of a sealed Waring Blendor jar in which the cheese was macerated in the presence of phosphoric acid. The sealed jar prevented the loss of the volatiles, and the acid accelerated the release of the sulfur compounds. A stream of oxygen-free nitrogen, sparged into the cheese slurry, carried the volatiles through a train of absorption traps. The system of differential absorption was the same as that of Dateo *et al.* (1957), which consisted of: one solid lead acetate [$Pb(OAc)_2$] for H_2S, two 4% mercuric cyanide [$Hg(CN)_2$] for mercaptans, and two 3% mercuric chloride ($HgCl_2$) for organic sulfides and disulfides. Thin-layer chromatographic separations and melting point data showed CH_3SH to be the sole mercaptan trapped from Trappist cheese volatiles (Grill *et al.*, 1966). Thus, any radioactivity recovered in the $Hg(CN)_2$ trap resulted from $CH_3{}^{35}SH$.

The injected portion of the cheese was cut from the wheel, the wax removed, and a 100-gram sample weighed and placed in the blender jar. After 90 minutes of aspirating the cheese, all traps were disconnected; the $Pb(OAc)_2$ was dissolved in 80 ml. of distilled water, and the contents of both $HgCl_2$ traps were combined. One milliliter of the trapping solution was pipetted into 16 ml. of scintillation liquid. The radioactivity of the cheese slurry in the blender was also measured using 1 ml. of sample to 16 ml. of scintillation liquid. To determine how much of the methionine injected in the localized area diffused throughout the cheese, the remainder of cheese without surface growth was made into a slurry and measured for radioactivity.

Determinations were made in duplicate and the data were corrected for background counts, half-life, and counting efficiency. The scintillation liquid was that devised by Bray (1960), with slight modification, for counting aqueous solutions in a liquid scintillation counter. One milliliter of hyamine 10-x (Rohm and Haas Co.) was added to every 15 ml. of Bray's scintilla-

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tion solution. Preliminary findings, using the above scintillation liquid which contained an aqueous solution of ^{35}S-methionine of known activity measured on a Packard Tri-Carb scintillation spectrometer, showed a counting efficiency of 26%.

Results

Members of the Dairy Department staff, on comparing samples from the two cheese groups, found the cheese which was waxed immediately after brining to have a bitter acid flavor; no trace of the Limburger-like character was detected. The texture of the cheese was not as smooth as a Trappist cheese cured under normal conditions, but it did show signs of proteolytic breakdown. The sample of cheese cured under normal conditions exhibited the flavor and texture characteristics of a typical Trappist cheese. Table I contains data on the degradation of ^{35}S-methionine into volatile sulfur compounds by Trappist cheese with and without the growth of surface organisms. Analysis of the cheese containing no surface flora showed that approximately one half of the methionine injected into the 100-gram section diffused throughout the cheese. In this cheese, insignificant levels of radioactive volatile sulfur compounds were derived from the radioactive methionine which remained in the 100-gram section of cheese. The low levels that were detected could have come from chemical rather than enzymatic breakdown of the methionine. The level of radioactivity detected in the $Hg(CN)_2$ traps containing CH_3SH from the cheese with surface flora was much greater (ca. 295,000 c.p.m.) than that detected for the cheese without surface flora. Activity was also slightly higher in the $Pb(OAc)_2$ and $HgCl_2$ traps, but it was still insignificant. In the case of the $Pb(OAc)_2$, incomplete flushing of CH_3SH from the trap may have caused the slight increase in radioactivity. The amount of radioactivity measured in the traps, the cheese slurry, and the remainder of cheese without surface growth represented about 94% of the radioactivity originally injected into the cheese.

Discussion

The results show that free methionine served as a precursor for CH_3SH but not for H_2S, and that the growth of surface microorganisms was necessary for the formation of CH_3SH. Tuckey and Sahasrabudhe (1957) have shown free methionine to be present in Brick and Limburger cheeses at levels ranging as high as 0.48 and 1.10 mg. per gram of cheese, respectively. Trappist cheese is the same type of cheese as Brick and Limburger in that it depends upon proteolysis for ripening. It ranges in flavor intensity somewhere between the other two. The authors feel it is safe to assume that free methionine also exists in Trappist cheese. The amount of free methionine reported for Brick cheese far exceeds that needed to provide the 2.3 μg. of CH_3SH per gram of cheese determined for Trappist cheese (Grill *et al.* 1966). There is still the possibility that CH_3SH may also be produced from cysteine and cystine. Tsugo and Matsuoka (1962) found only negligible amounts of CH_3SH produced from cysteine by an enzyme preparation of *P. caseicolum* isolated from white mold cheese. This may also be true in the case of the surface microorganisms found on Trappist cheese. More likely, cysteine and cystine are the precursors for the H_2S present in Trappist cheese since these results indicate that H_2S was not a significant breakdown product of methionine.

The high level of radioactivity absorbed in the $Hg(CN)_2$ traps from the cheese having surface growth clearly indicated that the normal growth of surface organisms was essential for the degradation of methionine to CH_3SH. Methionine degradation by any other means, such as the Strecker degradation as suggested by Keeney and Day (1957), appears to be of little importance in Trappist cheese.

The results presented herein complete a study which has shown the presence of CH_3SH, demonstrated its flavor significance (Grill *et al.*, 1966), and elucidated its mechanism of formation in a surface-ripened cheese.

Table I. Degradation of ^{35}S-Methionine[a] into Volatile Sulfur Compounds during 3 Days of Incubation within Two Wheels of Trappist-Type Cheese One with and One without the Growth of Surface Organisms

	^{35}S Recovered in Volatile Sulfur, C.P.M.	
	Without surface growth	With surface growth
Cheese slurry in blender	73,548,700	69,874,700
Remains of cheese wheel	73,919,500	...[b]
Trap 2 $Pb(OAc)_2$	2,640	7,120
Trap 3 $Hg(CN)_2$	1,740	291,600
Trap 4 $Hg(CN)_2$	960	6,160
Traps 5–6 $HgCl_2$	180	600

[a] 156,311,700 c.p.m. added to each wheel; data corrected for background and half-life. Each figure for c.p.m. represents the average of duplicate determinations.
[b] Not counted.

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