FLAVOR DEVELOPMENT AND MICROBIOLOGY OF SWISS CHEESE—A REVIEW

III. RIPENING AND FLAVOR PRODUCTION

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ABSTRACT

This paper, the third of four, discusses the ripening and flavor production processes of Swiss cheese. First, general ripening changes are reviewed; then, a discussion of eye formation and body and texture changes follows. The important flavor compounds found in Swiss cheese are examined in detail beginning with consideration of the analytical methods used to isolate and measure fatty acids. The different volatile and nonvolatile fatty acids and their importance in Swiss cheese are considered, as well as production of propionic and acetic acids from lactate, production of keto acids, and lipolysis. Then, analyses for and significance of carboxyl compounds, alcohols, esters, lactones, hydrocarbons, and diacetyl are presented. Possibilities for production of these compounds by microorganisms are given. Subsequent sections deal with nitrogenous compounds, degradation of caseins to peptides and amino acids and their importance to flavor, and end with a review of sulfur compounds present.

After Swiss cheese has been removed from the press, brined, and prestored for 7 to 14 days, it is transferred to the hot room where the temperature is 21 to 25°C (70 to 77°F). During the period in the hot room cheese becomes sufficiently elastic and the principal ripening of the cheese, with active growth of propionibacteria, takes place, forming the typical eyes of Swiss cheese (430). When the eyes are large enough, the cheese is moved to the curing cellar or "finished cooler" and kept there at 2 to 5°C (35.6 to 41°F) for 2 to 9 months. Ripening for at least 6 months should develop the fine, full flavor characteristic of a well-ripened Swiss cheese (116); however, most commercial Swiss cheese sold in the United States today is much younger when sold (345).

GENERAL ASPECTS OF SWISS CHEESE RIPENING

During the time in the press, high-temperature lactic-acid bacteria rapidly hydrolyze lactose in the young cheese to glucose and galactose (338), which, by the Embden-Meyerhof pathway, is oxidized to lactic acid by the same bacteria (8). Production of lactic acid in the cheese produces a readily assimilable substrate for the propionibacteria. Propionibacteria were first found by Sherman (393) to be essential for eye formation as well as production of the characteristic sweet flavor of Swiss cheese.

A large part of the lactic acid in Swiss cheese is produced by homofermentative lactic-acid fermentation, but a small portion also is produced through the heterofermentative pathway. Moderate amounts of acetic acid are found in the cheese after pressing (218). Because of this heterofermentative activity, carbon dioxide, which induces growth of propionibacteria, also has been found (150). Lactic-acid bacteria also reduce the redox potential of the Swiss cheese to −200 mv, which is favorable for growth of propionibacteria (199). Another important factor for growth induction of propionibacteria is the pH-value of the cheese out of the press. The pH must be between 5.0 and 5.3 to produce good Swiss cheese. If the pH is lower than 5.0, growth of propionibacteria will not take place (431).

During the hot room and curing room treatment of Swiss cheese, the number of S. thermophilus and L. helveticus decline (79), even though it has been stated that mixed cultures of S. thermophilus and L. helveticus may grow at temperatures as low as 15°C (59°F) (171). The primary reason for this numerical decline is the elimination of lactose as an energy source and the formation of some metabolic products in the cheese that may have an adverse effect on growth, especially as the cheeses are ripened near their minimum growth temperature. Lactobacillus casei, however, grows during ripening, and some investigators consider it an important ripening organism (79).

Growth of propionibacteria is induced in the hot room. Frazier and Wing (121), in 1931, stated that significant growth took place after about 3 weeks in the hot room, when eye-formation begins. After 2 weeks in the hot room, the population of propionibacteria had increased 3 to 980 times the corresponding numbers present in curd in the press (236). This

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number was dependent on the initial pH of the cheese out of the press. Maximum numbers of propionibacteria were found after 6 or 8 weeks when the initial pH had been between 5.15 and 5.37. With initially lower pH values, maximum population occurred between 10 and 13 weeks. The population of propionibacteria showed a positive correlation with pH from the press. After the maximum number of propionibacteria was reached and sufficient eye formation had occurred, the cheese was transferred to the cold room. The number of propionibacteria slowly decreased during further ripening in the cold room (79, 236), even though some strains of *Propionibacterium* can grow at temperatures as low as 7°C (44.6°F) (319).

The distribution of propionibacteria in conventional Swiss cheese with rinds has been determined (347). Very few propionibacteria (1 × 10⁷/g cheese) were found just below the surface of the cheese. Samples at 2.25 cm (7/8 inch) below the surface contained many more propionibacteria (74 to 1,200 × 10⁷/g cheese) than the exterior portion, and the number progressively increased toward the center of the cheese (410 to 2,200 × 10⁷/g cheese). Decrease of propionibacteria toward the exterior possibly results from such unfavorable conditions as lower moisture, higher salt concentrations, and higher redox-potential. Rindless Swiss cheese has a more uniform distribution of propionibacteria because of the alleviating influence of the impermeable wrapper.

During curing, diverse chemical changes are brought about in Swiss cheese because of glycolysis, lipolysis, and proteolysis. Lactic acid is degraded to propionic and acetic acid, and protein hydrolysis takes place. Flavor development depends on the relative amounts of lower fatty acids produced (245). Lower free fatty acids are related to the lactate content of the Swiss cheese, and lactate content is related to the lactose content in the cheese the day after manufacture. Cheese of good quality contains large amounts of propionic and acetic acid and small amounts of butyric and higher fatty acids. With higher amounts of butyric and higher fatty acids, Swiss cheese flavor is strong and rancid (245). Sahli and Lehmann (370) showed that a well-ripened Swiss cheese contained 0.3% (by weight) acetic acid and 0.4% propionic-acid but, also at least 0.012% butyric acid.

Proteolysis takes place during curing and the content of free amino acids in Swiss cheese increases. Free amino acids in amounts from 3.2 to 4.1 mg% were recognized as normal for Swiss cheese (143). Protein breakdown is delayed in Swiss cheese containing copper (190). In a study at Iowa State University on Iowa-style Swiss cheese, however, no retardation of proteolysis was observed, and, in most instances, higher proteolytic values were observed (Maurer and Reinbold, unpublished results). Propionibacteria do not affect proteolysis significantly (207). Most of the characteristic sweet flavor of Swiss cheese has been attributed by some workers (449) to the proline content, which is higher in Swiss cheese than in any other cheese. Other workers (296) believe that proline and the ratio of propionic acid to proline is important for Swiss cheese flavor. Besides these two important groups of compounds, a large number of volatile carbonyl compounds in low concentrations has been isolated, and these carbonyl compounds may be important for flavor (244).

The pH of the Swiss cheese rises in the cold room, the hot room, and the curing room. Cheese out of the press has a pH from 5.1 to 5.3, and these values rise about 0.05 to 0.1 pH unit in the cold room (116). The increase in pH in the hot room is much faster and the pH will be about 5.5 at the time the cheese is moved to the curing room (116). The pH after 2 to 3 months should not be less than 5.5 (447). If this pH is not reached, normal ripening does not occur. Sahli and Lehmann (370) reported that high-quality Swiss cheese has a pH value between 5.84 and 5.70.

The fat content in dry matter of a high-quality Swiss cheese should be about 48% (370). The distribution of fat in Swiss cheese was studied by Oeben and Ryser (299) who found that fat content varied if the cheese was sampled on the outside or in the middle of the wheel. Moisture content also varied with the sampling site in the cheese (299). Sahli and Lehmann (370) found average moisture in high-quality Swiss cheese to be about 36.9% at 3 months and 34.5% after 6 months. Rindless block Swiss cheese will contain perhaps 2.06% more moisture (345). Green cheese containing more than 39.7% moisture results in poor quality cheese (371). This detrimental effect is most evident in relationship to eye formation, and the defect known as “oversetting,” too numerous and small eyes, will be very pronounced (372). High moisture content may be the result of conditions in the manufacturing process such as low-fat milk, soft-curd milk, slow acid-development, low setting temperature, fine harping, low cooking temperature, and shortening of each step in the procedure (371, 372).

A high-quality Swiss cheese should contain eyes of proper size and form, and the eyes should be evenly distributed in the cheese. Normal eyes should have a diameter from 1.25 to 2.54 cm (0.5 to 1 inch) and should have 2.54 to 7.62 cm (1 to 3 inches) between them (141). Gas in the eyes of a normal cheese consists of carbon dioxide and nitrogen. Nitrogen is occluded from the air (90) and carbon dioxide is produced by propionibacteria. Because of the rela-
relatively few eyes produced in Swiss cheese, it is improbable that eyes are formed at the location of colonies (60). Gas, as it is produced, may diffuse through the curd and collect at weak points in the cheese where the eyes will be formed (60). The ripening of Edam cheese has been followed with X-rays (385). Eye formation could be followed but this technique was difficult to apply to large blocks of Swiss cheese. With smaller blocks of Swiss cheese, van Allemersch and Devogelaere (4) established, by X-ray analysis, that the eyes of Swiss cheese are usually visible after ripening at 13 °C (55.4 °F) for 12 days. The constant growth of the eyes can be clearly followed to the end of the storage period in the hot room at 20 °C (68 °F). A marked similarity was observed in all instances between the X-ray image and a corresponding section of cheese. Presence of numerous small openings detected by X-ray analysis in young cheese was most often associated with the texture defect "oversetting" (Vielsetzer) (4). Copper-containing cheeses were shown to have fewer and larger eyes than control cheeses, and, at higher copper concentrations (11 mg/kg), they required a longer ripening time for eye development. Velocity of carbon dioxide production was markedly reduced (Maurer and Reinbold, unpublished results).

Relative humidity in the ripening room also has an influence on eye formation. Uneven relative humidity caused uneven eye formation in Swiss cheese because of differences in rind resistance of the cheese (424). This observation, of course, would not apply to rindless block Swiss cheese cured in an impermeable wrapper (345). If cheese held at regular curing temperature showed evidence of too many or too large eyes, further swelling could be avoided by placing the cheese in a cold room at about 4 °C (39.2 °F) (18). The swelling effect could result from continued carbon dioxide production from propionibacteria in Swiss cheese at low temperatures. Park et al. (319) showed that strains of propionibacteria may grow at temperatures as low as 7.2 °C (45 °F).

During ripening of Swiss cheese, the body gradually becomes softer, less tough, and less elastic. These changes probably reflect the solubilizing properties of proteolytic enzymes (116). Watson (456), however, observed that production of a desirable soft texture in the cheese was not necessarily coincident with the highest degree of proteolysis, quite the opposite actually being true. Variation in the colloidal structure of cheese, as shown by water binding, must be important in body characteristics. Swiss cheese tends to become more firm and less pliable as it loses moisture (200).

Body characteristics of Swiss cheese are due to the nature and state of the colloidal nitrogenous sub-

stances (210). Elasticity of cheese is highly temperature variable. Texture varies, not only from cheese to cheese, but also within an individual cheese (211). Body characteristics of Swiss cheese were attributed by Koestler (212) to "hard nitrogenous substances" resulting from high cooking temperature, which gives the cheese, together with a relatively low moisture content, its characteristic properties. Cheese physical properties are determined not only by chemical transformations occurring during lactic and propionic-acid fermentation, but also by the inner structural relation of the cheese curd. Characteristic for Swiss cheese is a high degree of elasticity, moderate firmness of the body, and high water-binding capacity. Mocquot et al. (272) found that total and plastic deformation of Swiss cheese was highly correlated with temperature and moisture. Eye number and size increased as total and plastic deformation increased. Rheological studies of Iowa-style Swiss cheese containing different amounts of copper showed that copper content did not influence strength of the cheese but that an increase in copper content was highly correlated with an increase in elasticity and viscosity (Maurer and Reinbold, unpublished results).

Changes in Swiss cheese curd, observed using electron microscopy (366), seemed to support the belief that serum x-casein forms the initial linking element between casein particles. Further changes in the cheese curd during syneresis, pressing, salting, and ripening were accompanied by a progressive increase in homogeneity of the casein matrix and in deformation of fat globules. Bacteria were found mainly in capillaries and nodes that were probably filled with whey, surrounded by zones of protein breakdown, containing crystal aggregates, believed to be of the less soluble amino acids. For example, Flickiger and Schilt (113) found tyrosine produces white crystals in Swiss cheese.

**Flavor Development in Swiss Cheese**

Ripening of Swiss cheese is a slow process. Controlled, accelerated ripening would be of great help for flavor studies of Swiss cheese. Kristoffersen et al. (225), in 1967, developed a procedure for accelerated ripening of Cheddar cheese that involved use of a liquid cheese product, which acquired an intense, sharp, balanced Cheddar flavor in their judgment. Such factors as storage temperature, salt concentration, agitation, mineral-vitamin addition, and storage of frozen curd also were investigated (398). Singh and Kristoffersen later applied this method to Swiss cheese curd (398) and found that development of characteristic flavor was accompanied by increased
pH, decreased lactic acid, and formation of active sulphydryl groups (400). Head-space volatile compounds produced were quantitatively similar to those of aged Swiss cheese. Development of Swiss cheese flavor in the slurry was enhanced by reduced glutathione, was directly related to initial pH and amount of propionic-acid bacteria, and was inversely related to salt concentration. Swiss cheese curd manufactured only with S. thermophilus and propionibacteria, instead of L. helveticus, S. thermophilus, and propionibacteria adversely affected the flavor of the Swiss cheese slurry (400).

“Component balance theory”

Earlier belief was that only one or a few compounds caused Swiss cheese flavor. Propionic and acetic acid developed parallel to flavor development, and Babel and Hammer (18) proposed that propionic acid was the most important contributor to Swiss cheese flavor. Later, Virtanen and Kreula (449) attributed the characteristic sweet flavor of Swiss cheese to proline content. Hintz et al. (165) found that a minimum proline content of 2.0 mg/g and a minimum propionic acid content of 5.0 mg/g of cheese were necessary to produce a typical Swiss cheese flavor.

This concept has now generally changed and has been replaced by the “component balance theory” of Kosikowski and Mocquart (215), which states that a relatively small number of compounds are responsible for the differences in flavor of different cheese varieties. The more important components of cheese flavor include certain fatty acids (321), aldehydes (188), methyl ketones (152), diacetyl (52), amines (397), peptides (153), sulfur compounds (453), and amino acids (449); other compounds, such as esters (99), alcohols (125), partial glycerides (265), lactic acid (281), and salt probably also have some effect.

Neutral fat, para-casein, and moisture cannot be ignored since taste threshold concentrations of flavor compounds vary in fat and water solutions (321). These compounds are found in all types of cheeses to give them a basic cheese flavor, but it is the amount and proportion of a few typical compounds that produces the typical flavor of a certain cheese variety. Extreme shifts in proportions will lead to abnormal flavors. All components found in Swiss cheese may be considered important because of synergistic effects, even if they are found in lesser amounts than their threshold value. Other compounds present in amounts that are detected as off-flavors may still be essential for typical flavor when present in lower amounts (115).

Acids in cheese

Volatil fatty acids, fatty acids, and keto acids are always found in Swiss cheese. Volatile fatty acids probably are most important from the aspect of Swiss cheese flavor, but other acids may be important for their contribution to background flavor. In this part of the review, production and flavor of acids is discussed.

Analytical methods. With development of chromatographic methods, the number of reports concerning the presence of various acids in cheese has increased. In older literature, only reports about volatile fatty acids recovered through steam distillation are found (18, 310). Paper chromatography was first used for identification of volatile acids (166, 256), but Lindqvist et al. (256) found only propionic and acetic acid by the method they used. Harper (146), in 1953, developed a column chromatographic method with silicic acid as filling material for quantitative determination of acetic, propionic, and butyric acid in cheese. Samples were prepared by adding sulfuric acid to 5 g cheese to bring the pH to 1.7 to 2.0, and the samples were added directly to the column. Besides the volatile acids, pyruvic acid also was separated. Thin-layer chromatography also has been used for analysis of volatile fatty acids from Swiss cheese (16).

Gas chromatographic methods in particular were shown to be useful in analysis of volatile and nonvolatile fatty acids (36, 176, 291, 321, 421). Volatile acids could usually be separated from cheese by steam distillation of an acid cheese-slurry (321, 421), or an acid water-filtrate could be used (16). To determine higher fatty acids, Bill and Day (36) made a thick, acid, cheese slurry that was packed in a centrifuge tube and heated to 40 C (104 F), then it was centrifuged and the fat layer used for analysis. Fatty acids were methylated before gas chromatography was done. This method does not permit total recovery of fatty acids, but is most commonly used. Volatile and non-volatile fatty acids also can be isolated by extraction with organic solvents (239). A glass column seems preferable to a steel column for analysis for free volatile fatty acids (291, 314, 315).

Volatil and nonvolatil fatty acids in Swiss cheese. In 1904, Orla-Jensen (310) found that Swiss cheese contained propionic and acetic acid in the mol proportion 2.3 to 1. It was not until 1921, however, that Sherman (393) concluded that propionibacteria were necessary to ensure proper eye and flavor formation. As had been noted many times already, these bacteria produce propionic acid, acetic acid, and carbon dioxide from lactose and lactate (392).

Swiss cheese with a conspicuously sweet flavor was found by Babel and Hammer (17) to contain a higher volatile acid content than cheese lacking a definite sweet flavor. Addition of propionates to processed Swiss-type cheese lacking in flavor produc-
ed a sweet flavor. This suggests that added propionates have a positive effect on Swiss cheese flavor.

High-quality Swiss cheese contains relatively large amounts of acetic and propionic acid with little or no butyric acid and a small portion of higher fatty acids (217). Krett and Stine (217) observed that, in Swiss cheese with a flat flavor, the acetic-acid content was comparable to normal cheese but little or no propionic or butyric acid was present. Highly objectionable flavor was found in cheese containing large amounts of butyric acid. Krett et al. (218) determined the amounts of volatile fatty acids in Swiss cheese. In cheese coming out of the hot room, the amounts of fatty acids of the cheese in mg/100 g cheese were 167 acetic, 344 propionic, 18 butyric, and 30 higher volatile fatty acids. In 140-day-old cheese, the content was 290 mg acetic, 630 mg propionic, 38 mg butyric, and 50 mg higher fatty acids. Using Harper's method (146), Hintz et al. (165) found acetic, butyric, propionic, and fatty acids of C₆ and greater chain lengths in all cheeses, and valeric acid was observed in all cheeses except one. Typical values in an 11-month-old cheese of good quality were 3 mg acetic acid, 5 mg propionic acid, 1.1 mg butyric acid, 0.19 mg valeric acid, and 24.2 mg of the higher acids per gram of cheese. No correlation was found between free fatty acids and the age of the cheeses. Comparable values for these acids in Swiss cheese also were found by Kurtz et al. (236). The propionic-to-acetic acid ratio in cheese was found to be 0.3 to 1.0, but this amount of acetic acid may be high because pyruvic acid was eluted with acetic acid (236, 241). After assessing aqueous solutions of propionic acid and propionate, Kurtz et al. (236) stated that propionic acid did not seem to cause the sweet flavor in Swiss cheese, but that it still is an important factor in the flavor complex. This refutes the claim of Babel and Hammer (17), that propionic acid is responsible for the sweet flavor of Swiss cheese.

Schormüller and Langner (378) found the fatty acids shown in Table 1 in Swiss cheese by paper chromatography; C₆ and C₈ monocarboxylic acids were found only in small amounts. Patton (322) observed that Swiss cheese was characterized by a high proportion of propionic acid; he also detected 2- and 3-methylbutyric acids among the volatile fatty acids. n-Valeric acid was not detected, but Hintz et al. (165) who found this acid used Harper's method (146), which does not separate the branched C₆-acids from n-valeric acid. Schormüller et al. (379) also found high amounts of propionic and acetic acid in the "tear fluid" of Swiss cheese and other volatile fatty acids in lower amounts (Table 1). In a comparative study between Gruyère and Swiss cheese, the amounts of volatile acids seemed a little higher in Gruyère (239).

<p>| Table 1. Volatile and nonvolatile fatty acids in Swiss cheese compared with Cheddar, Tilsit, and Edam |
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<th>Acids</th>
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<th>Emmentalᶜ</th>
<th>A</th>
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<th>D</th>
<th>E</th>
<th>Tear fluidᵇ</th>
<th>Cheddarᵇ</th>
<th>Tilsitᵇ</th>
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ᵃAnalysis values per 100 g cheese.
ᵇHintz et al. (165).
ᶜSchormüller and Langner (378).
ᵈKlermeler et al. (193).
ᵉLangler and Day (243).
ᶠSchormüller et al. (379).
ᵍAnalysis values per 10 g tear fluid.
ʰBills and Day (36).
Langler (241) and Langler and Day (243) made detailed studies of the amounts of the volatile and other major fatty acids in Swiss cheese. Results are in Table 1, where the values for the free fatty acid composition of Cheddar cheese are included for comparison (36). High-quality Swiss cheeses, including two imported samples, were analyzed (241, 243). 2-Methylbutyric acid was detected in all cheeses and might possibly be important for flavor. Cheeses of the lowest quality also contained the lowest amount of this acid. 3-Methylbutyric acid seemed not important for the flavor complex since it was found in only two of the cheeses. The ratio of propionic to acetic acid indicated no simple relationship to flavor. Acetic, butyric, caproic, caprylic, and capric acid existed in Swiss cheese (241, 243) in amounts higher than their flavor-threshold values in water, milk, and oil as determined by Patton (323) and may, therefore, be of importance in flavor. By comparing the proportions of the esterified fatty acids of milk fat with those of the higher free fatty acids of Swiss cheese, the results suggest that these acids arise from a nonspecific hydrolysis of milk fat (241), which also is believed to apply to Cheddar cheese (36).

Propionic-acid production in Swiss cheese. For a detailed review of propionic-acid metabolism, the reader is referred to a review paper by Hettinga and Reinbold (157). In this review, propionic-acid formation will be discussed primarily in association with Swiss cheese.

It has been known since 1906 that propionic acid in Swiss cheese was formed by fermentation of lactic acid by propionibacteria (123). These organisms also may produce propionic acid from lactose, glucose, succinate, glycerol, pyruvic acid, and peptone, but the proportion of propionic acid to acetic acid varies with substrate (302, 445). It also is known that their metabolism advances through phosphate esters (412). So, it has been established, in general, that propionibacteria metabolize glucose and lactose through the Embden-Meyerhof scheme (21, 412). A reaction scheme for production of propionic and acetic acid from glucose and lactate, was proposed by Allen et al. (5); (see also reference 157.)

In Swiss cheese, propionic acid is usually formed from lactate produced by the high-temperature starter bacteria. Lactate promotes growth of propionibacteria in cheese, and El-Hagarawy et al. (101) found that addition of lactate to milk increased acid production. No relationship was found between maximum amount of propionibacteria and amount of propionic acid produced (102). With rapid growth in a broth medium, the proportion of propionic acid to acetic acid was about 2:1 for the first 9 days and 1:1 thereafter (100). By addition of propionate and acetate in a proportion of 2:1 to a slurry from Swiss cheese, growth of propionibacteria was inhibited (14). For normal Swiss cheese, the amounts of these acids are variable and, in many instances, too low to hinder late fermentation (Nachgärung). In good, nonperishable cheeses, the proportion of propionate to acetic averages about 1.98, and in perishable cheeses about 1.26.

Addition of nitrite (0.001 to 0.2%) to cheese milk inhibits propionic-acid fermentation in Swiss cheese (195). Addition of hydrogen peroxide (0.035 to 0.17%) to cheese milk, followed by catalase treatment lowers the production of propionic acid, but raises that of acetic acid and increases protein breakdown. Heating the milk [65 to 72 C (149 to 161.6 F) for 40 sec] inhibits propionic-acid fermentation in the cheese to a degree dependent on the severity of the heat treatment.

High-temperature lactic-acid starters produce some acetic acid (195) in the cheese before propionibacterial growth is induced, so, before the cheese is moved to the hot room, acetic acid is found in higher proportions in the cheese than propionic acid. Table 2 shows the production of acetic, propionic, and butyric acid in Swiss cheese during aging (195). By using S. thermophilus and L. helveticus as starter instead of just L. helveticus, the ratio of propionic to acetic acid was lowered (199). Decrease in lactic acid in cheese milk promotes the propionic-acid fermentation because the pH of the cheese is raised, showing that pH regulates production of propionic acid by propionibacteria. Dilution of lactose by addition of water during cheese production raises the ratio of propionic to acetic acid (199). Lactic-acid fermentation lowers the redox potential of the Swiss cheese to ~200 mv, and propionibacteria raise it to ~50 to ~100 mv during growth. Addition of metallic iron and its chlorides promotes the propionic-acid ferme-
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tation, even if it raises the redox potential (199, 200). The proportion of propionic to acetic acid is strongly influenced by the presence of ionic iron. Ferrous hydroxide addition produces propionic acid as the primary end product but ferric hydroxide produces mainly acetic acid (189).

The velocity of the propionic-acid fermentation depends on the bacterial producer of the lactic acid (201). *Streptococcus thermophilus* and *L. bulgaricus* showed no distinct differences, whereas the propionic-acid fermentation following growth of *L. lactis* is markedly slower. This depended on the lactic acid produced; as *S. thermophilus* produces the L (+) form, *L. bulgaricus* the L (+) D (-) form, and *L. lactis* the D (-) form of lactic acid.

Swiss cheese was made with either *S. thermophilus*, *L. helveticus*, or *L. lactis* as the high-temperature starter (407). Time of lactic-acid formation during pressing of the curd had a definite influence on the total amount of lactic acid formed in the cheese. *Streptococcus thermophilus* produced lactic acid during the first hours of pressing, while *L. lactis* was late and slow in acid production. Mainly L (+) lactic acid was found in the cheese made solely with *S. thermophilus* and this optical form of lactic acid was then utilized very rapidly. In cheese with *L. helveticus*, both L (+) and D (-) lactic acid were formed in about equal amounts, but the L (+) lactic acid decreased the fastest. With *L. lactis*, the amount of D (-) lactic acid was highest and it decreased slower during the hot room period (407). Cheese made with *S. thermophilus* and *L. lactis* alone were of inferior quality. This seems to support Kiuru’s observation on the relationship between optical form of lactic acid produced and the velocity of the propionic-acid fermentation (201).

Copper was found to have an inhibitory effect on propionibacteria (280 and Maurer and Reinbold, unpublished results). With about 17 mg copper/kg of dry cheese the formation of propionic acid was severely retarded (197, 198, 470). The lactic-acid content increased, and the content of volatile acids decreased, with increased copper levels in Swiss cheese (Maurer and Reinbold, unpublished results).

In an experiment with Swiss cheese produced in copper and steel vats, Steffen and Blanc (407) found that the total amount of lactic acid in cheeses from copper vats was lower than in cheeses from steel vats. This indicates that copper probably inhibits lactic-acid production by high-temperature lactic-acid bacteria (407). Using an enzymatic method, Steffen (406) measured the amount of L (+) and D (-) lactic acid produced. Both L (+) and D (-) lactic acid were found in lower amounts in cheese from the copper vats than in cheese from steel vats. In both kinds of cheeses, the amount of L (+) lactic acid was highest. In the cheeses made in a copper vat, the lactic-acid content started to decrease after 30 days, while in cheese from a steel vat, the L (+) lactic acid started to decrease after 3 days. The D (-) lactic acid reached its maximum value after 35 days before it started to decrease (407). This seems to indicate that L (+) lactic acid is used before D (-) lactic acid, and that small amounts of copper may inhibit use of L (+) lactic acid more than use of the D (-) isomer. In vitro experiments show that this was a result of the differing influence of copper on the activities of the D (-) and L (+) forms of the lactic dehydrogenase. L (+) lactic dehydrogenase was more inhibited than the D (-) form. Lactate, especially in the D (-) form, then tended to increase in cheese paralleling the rise in copper content (197, 198).

Lipolysis. Most volatile acids produced in Swiss cheese appear to be formed by the metabolism of bacteria. Schormiller (376) mentions that the small amounts of butyric acid found seem to come from the metabolism of clostridia. There are other sources that suggest that butyric acid and, especially, the higher fatty acids come from lipolytic breakdown of milk fat, because the proportions of free fatty acids other than propionic and acetic acid found in Swiss cheese closely resemble the proportions of fatty acids in milk fat (36, 86, 241).

Milk contains a lipase that will usually be inhibited by pasteurization (376). Consequently, the original lipase in milk seems of little importance in the ripening of cheese made from pasteurized milk. In Swiss cheese from raw milk and LTST heat-treated milk, however, the effect of the lipase is important (304, 376). If lipase is added to the pasteurized cheese milk, lipolysis will liberate higher amounts of fatty acids than in the control vat (304), so the milk lipase is not inactivated during cheesemaking as was assumed by Bachmann (19).

Microbial lipases may have an important effect on cheese flavor. Generally, active lipase production is associated with psychrotrophic bacteria. Representatives of these organisms belong to the genera *Pseudomonas*, *Alcaligenes*, *Achromobacter*, and *Serratia*. Microorganisms growing on the cheese surfaces are especially active because these genera are usually strictly aerobic, but their lipase may still be effective in the center of the cheese (376). Pasteurization destroys the lipase-carriers of raw milk, but the enzymes are not usually inactivated (405). In a study of Swiss cheese produced from milk inoculated with lipase-producing pseudomonads and thereafter pasteurized, Finheiro et al. (333) found a significant increase in
free fatty acids in the cheeses that was caused by residual lipase.

No extracellular lipase has been found in lactic-acid bacteria. Nakanishi et al. (285) detected no appreciable lipase activity in six strains of starter bacteria, and the formation of free fatty acids in cheese was not attributed to lipase activity from starter strains. Later, however, it was shown that lactic streptococci contain intracellular lipases that hydrolyze tributyrin (125). Lactobacillus casei, which often is present in high numbers in Swiss cheese during ripening, was shown by Peterson and Johnson (330) to contain intracellular lipases that are active at pH 5 to 6, covering the correct pH range for Swiss cheese during ripening. The lipases of eight strains liberated n-butyric, caproic, caprylic, and capric acids; two strains produced n-butyric, caproic, and caprylic acids (330). Lactobacillus casei in cheese was shown to increase the relative amounts of volatile fatty acids.

The lipase activity of propionibacteria has not been studied in detail. Werner (459) found no extracellular lipase activity in eight strains. Cantoni et al. (33) demonstrated, however, that six strains could produce butyric acid from tributyrin and free fatty acids from butterfat. Oterholm et al. (313) established that the lipase of P. shermanii was an intracellular enzyme, and may have some influence on the ripening of Swiss cheese. If this surmise is correct, the effect must be minimal in view of the well-established relationship between proportionality of volatile fatty acids and desirable Swiss cheese flavor.

Production of acids other than fatty acids. Table 3 shows some of the other acids observed in Swiss cheese. The first to study the keto acids of Swiss cheese were Bassett and Harper (22, 23). The content of keto acids was not essentially affected by the age of the Swiss cheese. All cheeses contained pyruvic, \( \alpha \)-ketoglutaric, \( \alpha \)-acetolactic, oxaloacetic, and oxaloacetic acids with pyruvic and \( \alpha \)-ketoglutaric as the main compounds present. In cultures of L. bulgaricus and S. thermophilus, similar patterns of keto acids were obtained but with disproportionately larger amounts of \( \alpha \)-ketoglutaric acid. If P. shermanii was included in the mixed culture, the pattern corresponded to that of the cheese (22). Keto acids of Swiss cheese also have been studied by Kreula and Virtanen (219) who detected \( \alpha \)-ketoisovaleric acid and \( \alpha \)-ketocaproic acid in addition to those found by Bassett and Harper (23). In tear fluid, only pyruvic, \( \alpha \)-ketoisovaleric, and \( \alpha \)-ketoglutaric acid were found (219). Schormüller and Langner (378) found p-hydroxyphenyl pyruvic acid in addition to the other keto acids in Swiss cheese. Lactic, succinic, fumaric, and malic acid also have been detected (378). The same acids have been found in the tear fluid of Swiss cheese (379).

As mentioned earlier, propionic acid usually arises from lactate, but some propionic acid may be produced from alanine, serine, or aspartic acid (376). A portion of the acetic acid also could be a metabolic product of citrate breakdown by propionibacteria (8). Succinic and pyruvic acid are intermediates in the breakdown of carbohydrates and lactate (376). Swiss cheese older than 7 months shows no interrelations between ripening time and pyruvic acid content (191). Cheeses with low copper content, however, contain less pyruvic acid than cheeses with a higher copper content. This may indicate that copper inhibits emptying of the pyruvate pool. This effect of copper will be discussed in more detail in the section dealing with diacetyl in Swiss cheese.

TABLE 3. ORGANIC ACIDS IN SWISS CHEESE COMPARED WITH CHEDDAR, TILSIT, AND EDAM CHEESE

<table>
<thead>
<tr>
<th>Acids</th>
<th>Domestic Swiss*</th>
<th>Emmenthal*</th>
<th>Emmental*</th>
<th>Emmental Tear Fluid</th>
<th>Cheddar*</th>
<th>Tilsit*</th>
<th>Edam*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>-</td>
<td>-</td>
<td>590.1</td>
<td>130.68</td>
<td>-</td>
<td>0</td>
<td>649.8</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>-</td>
<td>-</td>
<td>123.9</td>
<td>95.46</td>
<td>-</td>
<td>59.3</td>
<td>225.7</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>-</td>
<td>-</td>
<td>0.58</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malic acid</td>
<td>-</td>
<td>-</td>
<td>25.52</td>
<td>13.47</td>
<td>-</td>
<td>17.1</td>
<td>55.2</td>
</tr>
<tr>
<td>Oxaloacetic acid</td>
<td>Trace</td>
<td>+</td>
<td>+</td>
<td>0.62</td>
<td>Trace</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glyoxylic acid</td>
<td>Trace</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>+ + + +</td>
<td>+</td>
<td>9.08</td>
<td>4.49</td>
<td>+ + +</td>
<td>10.74</td>
<td>11.76</td>
</tr>
<tr>
<td>( \alpha )-Ketoglutaric acid</td>
<td>+ + +</td>
<td>+</td>
<td>1.46</td>
<td>1.32</td>
<td>+ +</td>
<td>7.07</td>
<td>8.40</td>
</tr>
<tr>
<td>p-Hydroxyphenylpyruvic acid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \alpha )-Ketobutyric acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \alpha )-Acetolactic acid</td>
<td>+ + +</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Bassett and Harper (23).
*Kreula and Virtanen (219).
*Schormüller and Langner (378).
*Schormüller et al. (379).
Amino acids are of some importance in the formation of different organic acids. Transamination and deamination of amino acids are specific enzymatic reactions of great importance. According to Virtanen and Tarnanen (451), propionibacteria form asparaginase, which decomposes aspartic acid into fumaric acid and ammonia. Ellfolk (103, 104) has isolated this enzyme and examined its mechanism. Other acids, such as α-ketoglutaric acid, α-ketobutyric acid, n-hydroxyphenyl pyruvic acid, and pyruvic acid, usually are formed by transamination of amino acids, while malic acid and oxaloacetic acid may be formed by deamination (376). On the other hand, amino acids may be formed by the same reactions because these enzymatic reactions are reversible, and it is the need of the microorganism that decides which way the reaction will go. Harper (148) showed that amino acids were produced from keto acids by L. bulgaricus and S. thermophilus.

Protein hydrolysate in a milk medium stimulated the production of volatile fatty acids by S. lactis (284). As stated in another paper, Nakae and Elliott (282) found that both lactic streptococci and lactobacilli produce fatty acids from casein hydrolysate. Streptococcus diacetilactis produces acetate from alanine, glycine, and serine, propionate from threonine; isobutyric acid from valine, and isovaleric acid from asparagine, asparagine, and serine, propionate from threonine, and isovaleric acid from isoleucine and leucine. The reactions were ascribed to oxidative deamination (283). A strain of L. casei produces the same volatile acids from amino acids as usually does Streptococcus diacetilactis (283). Lactobacillus casei is found in Swiss cheese, and these reactions may be the source of some of the volatile fatty acids of Swiss cheese. Nothing is known about the production of volatile fatty acids from amino acids by propionibacteria, although Ritter and Hämmi (359) found that isobutyric acid and isovaleric acid in Swiss cheese could be produced by micrococci from valine and leucine.

Other volatile carbon compounds

In this section, the significance of carbonyl compounds, alcohols, esters, lactones, and hydrocarbons in Swiss cheese will be discussed in detail. Little work in this field has been done with Swiss cheese, and the only detailed study was done at Oregon State University by Langler (241) and Langler et al. (244); consequently, many of the references in this section will necessarily refer to work done with other cheeses.

Analytical methods. Few studies were attempted involving volatile carbon compounds and their importance in cheese flavors before the development of chromatographic methods, and, especially, gas chromatography. Only in the last 10 years has understanding of the importance of these compounds developed. Isolation of these compounds from cheese and other food products without loss of volatiles was so difficult that Weurman (460) suggested that working at subzero temperatures under N2 atmosphere might be important to prevent chemical and enzymatic side reactions.

Volatile compounds are notable for their elusive character, their presence in minute amounts, and their unstable nature. They, therefore, present the analyst with a considerable problem in isolation and identification as unaltered flavor components. Usually, techniques such as solvent extraction and atmospheric or vacuum distillation are used before gas chromatography, but, recently, direct analysis of cheese or foods by "head-space vapor analysis" or "on-column trapping" has been used (250, 275, 460).

Steam distillation of cheese slurry was one of the first methods tried and was used by Walker and Harvey (454), who estimated amounts of n-saturated aldehydes and ketones up to C6 in Cheddar cheese by reaction with 2, 4-dinitrophenylhydrazine in an acid solution under anaerobic conditions (246). The usual method is to isolate cheese fat, containing the major portion of the volatiles, by centrifuging a mixture of cheese and water at about 40 C (104 F) (75, 252). The isolated fat is then either extracted or distilled. A recycling gas-liquid extraction apparatus in which a sweeping gas is recycled and pushes volatiles into a refrigerated trap has been developed (288). Another method using countercurrent contact of the milk fat with steam in a column, followed by other extraction of the condensed steam also has been used (54). The usual methods, however, use either a simple vacuum distillation method, which is especially effective for aldehydes (247), or a high-vacuum apparatus for isolation of all volatiles (50). A low-temperature low-pressure distillation for volatiles in Cheddar cheese has been used (250), and Langler et al. (244) used the same method for Swiss cheese. In this method, flavor volatiles are distilled into liquid nitrogen traps of glass, which can be fitted with a hypodermic needle that inserts the sample into the gas chromatograph. For identification, the different chromatographic peaks may be isolated by a trap and then used for mass spectral analysis (250, 271) or admitted directly to the rapid-scan mass spectrophotometer (75). Direct injection of the cheese oil into the gas chromatograph also has been used for analysis of cheese volatiles (252).

Extraction also may be used for isolation of volatile compounds before gas chromatography. For example, finely grated Cheddar cheese has been mixed with Celite 545 and packed into a column that was eluted with redistilled acrylonitrile (466). The first few drops were sufficiently concentrated to permit gas chromatographic analysis without solvent evapora-
tion. Recovery of added benzoic acid was 103%. Liebich et al. (253) determined the volatile compounds in Cheddar cheese by using different isolation methods: (a) centrifuging to separate the oil from the cheese, (b) low-temperature vacuum distillation of the cheese followed by extraction of relevant fractions with diethyl ether, (c) treatment of whole cheese as in (b), and (d) extraction of cheese oil with methanol. Oil from (a) and extracts from (b), (c), and (d) were analyzed by gas-liquid chromatography, and extracts were further analyzed for identification of compounds by combined gas-chromatography-mass spectrometry. More than 130 components were found by gas chromatography of Cheddar cheese, of which 120 were identified.

Large amounts of cheese are needed for distillation and extraction methods. The analysis of head-space gas samples, on the contrary, uses rather small samples. A method consisting of drawing gas from a column into the body of a hypodermic syringe containing cheese between Lintine filter discs, sealing the needle, heating to 90 C (194 F) for 8 min, and injecting the mixture of gases, including low boiling components into the column has been described (227). The method was empirical but fairly reproducible, and was used to compare the peak profiles of 13 different cheeses each of which showed a different profile. Injection of volatiles in the form of head space vapor into the column facilitated analysis and rendered less liable decomposition of the volatile components (317). Morgan and Day (275) felt that the headspace vapor method lacked precision and used the technique of on-column trapping of entrained volatiles, as described by Hornstein and Crowe (168). Samples were treated with sodium sulfate to saturate the aqueous portion, and the volatile matter was entrained with 50 to 100 ml nitrogen at 60 to 90 C (140 to 194 F), which gave sufficient material to detect minor amounts. This method was used by Langler for analysis of volatiles in Swiss cheese (241).

Besides the gas chromatographic method, colorimetric methods for specific compounds have been developed including a method to determine carbonyl compounds by use of dinitrophenyl hydrazines (279). Different colorimetric methods have been developed for diacetyl (56, 172, 249, 316, 396). Methods for formaldehyde and acetaldehyde also have been worked out (258, 273, 428).

**Table 4. Volatiles found in Swiss cheese (241)**

<table>
<thead>
<tr>
<th>Alcohols</th>
<th>Hydrocarbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ethanol</td>
<td>1. Toluene</td>
</tr>
<tr>
<td>2. 1-Propanol</td>
<td>2. 1-Octene</td>
</tr>
<tr>
<td>3. 1-Butanol</td>
<td>3. Nonane</td>
</tr>
<tr>
<td>4. 2-Pentanol</td>
<td>4. Ethyl benzene</td>
</tr>
<tr>
<td>5. trans-2-Hexene-1-ol</td>
<td>1.2; 1.3- or 1.4-Dimethylbenzene</td>
</tr>
<tr>
<td>6. 2-Phenylenethanol</td>
<td>5. Nonene</td>
</tr>
<tr>
<td>7. 2-Butanol</td>
<td>6. Dodecane</td>
</tr>
<tr>
<td></td>
<td>7. Benzaldehyde</td>
</tr>
<tr>
<td></td>
<td>8. Phenylacetaldehyde</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aldehydes</th>
<th>Lactones</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acetaldehyde</td>
<td>1. γ-Valerolactone</td>
</tr>
<tr>
<td>2. Propanal</td>
<td>2. β-Octalactone</td>
</tr>
<tr>
<td>3. Butanal</td>
<td>3. 8-Decalactone</td>
</tr>
<tr>
<td>4. Ethyl propionate</td>
<td>4. γ-Dodecalactone</td>
</tr>
<tr>
<td>5. Ethyl butanolate</td>
<td>5. 8-Dodecalactone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethers</th>
<th>Methyl ketones</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ethyl formate</td>
<td>1. Acetone</td>
</tr>
<tr>
<td>2. Methyl acetate</td>
<td>2. Butanone</td>
</tr>
<tr>
<td>3. Ethyl acetate</td>
<td>3. 2-Pentanone</td>
</tr>
<tr>
<td>4. Ethyl propionate</td>
<td>4. 2-Hexanone</td>
</tr>
<tr>
<td>5. Ethyl butanolate</td>
<td>5. 2-Heptanone</td>
</tr>
<tr>
<td>6. Butyl acetate</td>
<td>6. 2-Nonanone</td>
</tr>
<tr>
<td>7. 3-Methylbutyl acetate</td>
<td>7. 2-Undecanone</td>
</tr>
<tr>
<td>8. Methyl hexanoate</td>
<td>8. 2-Tridecanone</td>
</tr>
<tr>
<td>9. Ethyl hexanoate</td>
<td>9. 2-Pentadecanone</td>
</tr>
<tr>
<td>10. Methyl benzoate</td>
<td></td>
</tr>
<tr>
<td>11. Methyl octanoate</td>
<td></td>
</tr>
<tr>
<td>12. Ethyl octanoate</td>
<td></td>
</tr>
<tr>
<td>13. Methyl decanoate</td>
<td></td>
</tr>
<tr>
<td>14. Ethyl decanoate</td>
<td></td>
</tr>
<tr>
<td>15. 3-Methylbutyl octanoate</td>
<td></td>
</tr>
<tr>
<td>16. Ethyl dodecanote</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Miscellaneous compounds</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diacetyl</td>
<td></td>
</tr>
<tr>
<td>2. Dimethyl sulfide</td>
<td></td>
</tr>
<tr>
<td>3. Methyl vinyl ether</td>
<td></td>
</tr>
<tr>
<td>4. Chloroform</td>
<td></td>
</tr>
<tr>
<td>5. α-Phenene</td>
<td></td>
</tr>
<tr>
<td>6. α-Fenchene</td>
<td></td>
</tr>
<tr>
<td>7. o-Dichlorobenzene</td>
<td></td>
</tr>
<tr>
<td>8. 1,2,4-Trichlorobenzene</td>
<td></td>
</tr>
<tr>
<td>9. Benzothiazole</td>
<td></td>
</tr>
<tr>
<td>10. Disobutyl adipate</td>
<td></td>
</tr>
</tbody>
</table>

**Volatiles and production of volatiles in Swiss cheese.** Before Langler’s work in 1966 (241, 243, 244) very little research had been done in regard to volatiles in Swiss cheese. Acetoin and diacetyl were found in Swiss cheese in 1941 by Csiszar et al. as reported by Tomka (435). In 1958, Bassett and Harper (23) isolated these compounds from Swiss cheese: acetaldehyde, acetone, 2-pentanone, diacetyl, acetyl-methyl carbinol, and a five-carbon and a seven-carbon aldehyde or ketone. As shown in Table 4, Langler (241) identified 66 compounds in Swiss cheese. The amounts of some selected compounds are shown in Table 5 (244). These compounds will be discussed by the groups to which they belong:

I. Alcohols. Usually the amounts of alcohols found in Swiss cheese are too low to be believed to have any direct influence on flavor. Indirectly, however, they may contribute to flavor because of their ability to form esters with fatty acids. It should be mentioned that propyl esters have not been found (241). Major alcohols present are ethanol and 1-propanol. Ethanol is a common terminal endproduct.
in the breakdown of glucose (72), but the mode of formation of the methanol that has been found is hard to explain. Keenan et al. (187) demonstrated that S. lactis, S. cremoris, and S. diacetilactis produce ethanol, although S. diacetilactis produces it only in small amounts. These results were confirmed by Bassette et al. (25) for S. lactis, but not for S. diacetilactis. The same workers (25) also found that L. acidophilus produces ethanol. 1-Propanol and 1-butanol might be expected to result from reduction of their corresponding aldehydes. Lactobacillus brevis, L. casei, Lactobacillus plantarum, and L. lactis have been observed to produce ethanol and 1-propanol from acetaldehyde and propionaldehyde (185). Lactobacillus brevis also produces 2-butanol from butanone. A strain of L. brevis produced 1-propanol as a natural metabolite in cheese. Three strains of P. shermanii were shown to produce ethanol and 1-propanol (185). The reduction of 2-pentanone to 2-pentanol by Swiss-cheese starter organisms has never been observed, but mold and yeasts may reduce 2-pentanone to 2-pentanol (6). The film yeasts formerly used with Swiss-cheese starters may have been a source of 2-pentanol (116). Trans-2-hexene-1-ol was found in Swiss cheese. The source for this may be trans-2-hexenal, which has been found in the grassy aroma constituents of green forage (277). This compound may be reduced to the alcohol during ruminant metabolism and, thereafter, transported to the mammary gland. Another possibility for production would be by autoxidation of linolenate (277). 2-Phenyethyl alcohol also has been found in Blue cheese (73), and this compound imparts desirable character to a synthetic blue cheese flavor mixture (241). This compound may possibly be formed via degradation of phenylalanine and may be of some importance in Swiss cheese flavor.

II. Aldehydes. Acetaldehyde is the most common aldehyde found in fermented dairy products and cheeses (38, 74, 257, 286, 454). Acetaldehyde as a common carbonyl compound in Swiss cheese was first established by Bassett and Harper (23). The average concentration in Swiss cheese is close to 1.4 mg/kg (241). The flavor threshold of acetaldehyde is 0.4 mg/kg (151). Amounts of acetaldehyde in Swiss cheese (241) have been compared with amounts found in good-flavored butter cultures (257) and, on this basis, it has been assumed that acetaldehyde is an important factor in Swiss-cheese flavor.

Acetaldehyde is produced chiefly by bacterial metabolism. Production of acetaldehyde by 11 strains of S. lactis and S. cremoris was established by Harvey (151). No other aldehydes were formed. Streptococcus lactis, S. lactis var. maltigenes, S. cremoris, and S. diacetilactis produce amounts of acetaldehyde well over the flavor threshold in whole-milk media, and excessive amounts of acetaldehyde result in "green" flavor (260). Some of the acetaldehyde produced, however, would be removed by prolonged incubation (187). It is claimed that Streptococcus thermophilus produces more acetaldehyde than other homofermentative streptococci (44). Streptococcus thermophilus also produces acetaldehyde under anaerobic conditions (417).

Lactobacillus casei grown in milk produces acetaldehyde, but after 4 days the acetaldehyde disappears (25). Lactobacillus casei, L. brevis, and L. plantarum produce acetaldehyde slowly at temperatures close to cheese ripening temperatures (185). Three strains of P. shermanii have been shown to produce acetaldehyde and propionaldehyde (169). Different strains of starter organisms may produce different amounts of acetaldehyde, which may be the cause of off-flavors produced by some starters (409).
The other aldehydes found in Swiss cheese seem of less importance as flavor compounds. The branched chain aldehydes may possibly be intermediates in the production of fatty acids from amino acids (260). Phenylacetaldehyde may be formed by deamination and decarboxylation of phenylalanine. Benzaldehyde and phenylacetaldehyde exist in the volatiles of grass and corn silage, which may be another explanation of their occurrence in cheese (257).

III. Esters. Ethyl butanoate and methyl hexanoate are the primary esters found in Swiss cheese. Esters may influence the flavor of cheese, and if they are found in amounts higher than their threshold value, a fruity flavor may be recognized (37). The presence of esters in Swiss cheese is assumed to be due to the existence of free fatty acids and alcohols (241). Whether they are formed by simple mass action or enzymatic reactions is unknown.

IV. Hydrocarbons. Aromatic hydrocarbons have been found in Cheddar and Blue cheese, but not alkyl hydrocarbons, which in Swiss cheese possibly arise from packing material (241). Toluene also has been found in Swiss cheese, and did not seem to be an artifact introduced by distillation. The terpene, a-pinene, although present, was probably introduced into the milk from the forage eaten by the cow. None of the hydrocarbons seem important for flavor.

V. Lactones. δ-Lactones and γ-lactones were found in both Swiss and Cheddar cheese (241, 305). The concentration of lactones in Swiss cheese is very low and probably of no importance in flavor. Lactones are normal constituents of milk fat (40). δ-Lactones also may be produced by heating of milk (375). γ-Valerolactone probably is not a normal constituent in milk. It may be produced by microorganisms, and Boldingh and Taylor (40) mention various microorganisms that may convert keto-acids to corresponding lactones.

VI. Methyl ketones. A homologous series of methyl ketones was found in Swiss cheese (241). Their concentrations varied considerably between different cheeses. The presence of methyl ketones in heat-treated milk fat was determined by Langler and Day (242). The same compounds have been found in heat-treated milk, dry whole milk, and evaporated milk (320, 375, 467). These compounds could, therefore, be expected in Swiss cheese that is cooked at a high temperature (72). For example, methyl ketone concentrations have been reported to be highest in imported Swiss cheese, which might imply a higher cooking temperature or a longer cooking period (241, 244). Milk fat contains sufficient precursor to explain the level in Swiss cheese, and water is necessary for methyl ketone formation. The 2-heptanone level seems quite close to the threshold value. Methyl ketone mixtures have been observed to have a synergistic effect (242), so it is probable that methyl ketones affect the flavor of Swiss cheese (241).

Other sources of methyl ketones in microbial metabolism may not be ruled out. Methyl ketones are produced by molds used for Blue cheese production (72), but the starter organisms used for Swiss cheese have never been observed to produce methyl ketones. Development of methyl ketones in Cheddar cheese has been followed by Harvey and Walker (152). In 1-day-old cheeses, they found acetone, 2-butanone, and 2-pentanone; after 2 to 4 weeks, 2-heptanone was found, and after 20 weeks, 2-nonanone. The flavor of mature cheese was apparent after 8 to 12 weeks with an increase in 2-pentanone and 2-heptanone. This may indicate microbiological production of methyl ketones. Seven strains of S. lactis and S. cremoris have been shown to produce acetone (151). Production of acetone in milk cultures by S. lactis, S. cremoris, and S. diacetilactis has also been reported (184). Reports of the production of methyl ketones by high-temperature lactic-acid bacteria or propionic-acid bacteria could not be found.

VII. Miscellaneous compounds. The major portion of this discussion will be concerned with diacetyl. Some compounds found in minor amounts in Swiss cheese by Langler (241), however, deserve prior mention. Benzothiazole was recovered from Swiss cheese. Chlorinated aromatic compounds also were isolated but they might have been solvent contaminants. They also may originate from herbicides. Chloroform and di-isobutyl adipate also were isolated from Swiss cheese.

Csizsar et al. (64) found an average of 2.6 mg diacetyl/kg Swiss cheese, but they did not observe any relationship between this compound and the flavor of Swiss cheese. An average of only 0.8 mg/kg in Swiss cheese, however, was found by Langler (241). The threshold value of diacetyl is dependent on the medium in which it exists (27, 155) and the level of diacetyl in Swiss cheese is well over the threshold value and is considered an important part of the flavor (241).

Diacetyl is usually regarded as a by-product of citrate fermentation or it also can be obtained from carbohydrate metabolism. A scheme for the synthesis of diacetyl from citric acid has been prepared by Lindsay (257). Alpha-acetolactic acid was shown to be an essential intermediate in the synthesis of diacetyl (22, 181, 457), and pyruvate is an intermediate.

In cheese containing copper, the amount of pyruvate increased with the amount of copper in the cheese (191). The amount of diacetyl and acetoin also decreased with increasing amounts of copper.
The enzyme pyruvate decarboxylase is inhibited (193), and it seems that copper inactivates the participation of thiamine in the decarboxylation of pyruvate (194). Acetoin and diacetyl are formed by two different enzymes, and Collins (62) showed that acetoin could be formed from diacetyl but the opposite reaction does not occur. The earlier belief that this reaction was reversible (240) was shown to be incorrect (62).

The microbial flora of Swiss cheese is complex, and diacetyl could be produced by the heterofermentative species in mixed-strain lactic cultures (141). *Streptococcus thermophilus* was found to produce more diacetyl than other homofermentative lactic streptococci (44). The enzyme diacetyl reductase, which produces acetoin is found in *S. lactis* and *S. cremoris* (388). Keenan and Lindsay (180) found that *L. casei* and *L. plantarum* accumulated detectable amounts of diacetyl at 8 C (46.4 F). Branen and Keenan (45) observed that *L. casei* contained the enzyme diacetyl reductase. *Lactobacillus casei* showed the greatest ability to convert pyruvate to diacetyl and acetoin during late logarithmic growth; the optimum pH for diacetyl and acetoin production was in the range 4.5 to 5.5, which indicates that this conversion may take place in cheese (47).

Two strains of rod-shaped propionibacteria that produced acetoin and diacetyl in sterile milk and sterile whey were found by Tomka (435). The maximum level of acetoin was noted during the first few days of growth and remained constant for 15 days. A comprehensive study of acetoin and diacetyl production by propionibacteria was made by Antilla (10) who found that these compounds were produced from pyruvic acid with \( \alpha \)-acetolactic acid as an intermediate, as for other diacetyl-producing bacteria. In a detailed study by Lee et al. (248), a strain of *P. shermanii* was found that produced relatively large amounts of diacetyl. The diacetyl accumulation was greater at 21 C (69.8 F) than at 35 C (89.6 F) or 37 C (98.6 F), although the production was faster at the higher temperatures. Rapid cooling after incubation stabilized the level of diacetyl. From pH 4.0 to 4.5 was the most favorable range for diacetyl biosynthesis. Little or no diacetyl reductase was found in *P. shermanii* (248).

According to Emilsson and Sjöström (106), propionibacteria cannot ferment citric acid in cheese, however, these observations may have been due to the slow growth of the specific strain used in the study (8). Hietaranta and Antilla (164) observed that high-grade Swiss cheese contained only traces of citric acid and that citric acid disappeared during maximum growth of propionibacteria. In another report, Hietaranta and Antilla (163) reported that *P. peterssonii* fermented citric acid more rapidly than *P. pentosaceum* and that the utilization of citric acid was repressed by lactic acid.

### Nitrogen compounds

Considerable attention has been devoted to the importance of amino acids in Swiss cheese. Some workers attribute major significance in flavor production in Swiss cheese to amino acids. Others regard the influence of amino acids on Swiss cheese flavor with greater reservation. Amino acids, doubtless, have an influence on cheese flavor, and a high glutamic-acid content has been shown to have a direct influence in this regard (147). Even peptides seem to have taste-forming influences (414). Proteins, on the contrary, induce no flavor but are important for the body and texture of cheese. In this section, amino-acid content and its influence on flavor and the biochemical production and degradation of amino acids in Swiss cheese will be discussed.

**Amino acids in Swiss cheese.** Virtanen and Kreula (449) studied the relation of Swiss cheese flavor to the amino acids present in cheese and found that proline and hydroxyproline had a sweet taste and constituted a high proportion of the amino acids in the cheese serum. They, therefore, concluded that proline might be the cause of the sweet flavor. The high arginine content of casein also was thought to be another factor influencing flavor, but during ripening, 66% of the arginine was lost, so the disagreeable flavor of arginine had no effect (449). Two-thirds of the nitrogen compounds of tear fluid of Swiss cheese consisted of free amino acids and one-third of peptides and proteins. Proline was abundant in the tear fluid (450). Storgård and Hietaranta (413) considered that a portion of the flavor was due to the higher amounts of glutamic acid and aspartic acid in Swiss cheese than in other hard, rennet cheeses. The amount of acidic amino acids increased and the amount of basic amino acids decreased during storage of Swiss cheese.

The first qualitative study of amino acids in Swiss cheese was done by Block (39) in 1951. All the amino acids present in casein, as well as different degradation products of amino acids, were found using paper chromatography. Storgård and Lindqvist (414, 416) also studied the amino acids of cheese quantitatively. They determined that the amount of proline in Swiss cheese was higher than in any other cheese. The quantitative amount of amino acids in Swiss cheese was determined by paper chromatography by Kosikowski and Dahlberg (214) and Hintz et al. (165). Antila and Antila (15), and Ritter et al. (361) measured the amino acid content using more
exact ion-exchange chromatographic systems. The amino acid content of Swiss cheese tear fluid was measured by Schormüller et al. (379). These results are found in Table 6 and are compared with the amino-acid content of Edam and Cheddar cheese.

Not all amino acids are present in various Swiss cheeses and amounts vary widely among cheeses (165). Proline showed the widest quantitative variation, from 0 to 5.8 mg/g cheese. Amounts of cysteic acid, tauroine, proline, lysine, and histidine tended to increase during storage, but the amount of glycine tended to be constant. Amounts of aspartic acid, threonine, serine, glutamic acid, tyrosine, phenylalanine, and tryptophan present could not be related to the age of the cheese. A relationship between proline and propionic-acid content and flavor was believed to exist although Jäger (175) found no correlation between amino acids and flavor. The work of Antila and Antila (15) and Ritter et al. (361) showed that there were distinct differences between Finnish and Swiss Emmentaler. The proportion of glutamic acid to proline in Swiss Emmentaler was 2:1; in the Finnish cheese, it was 2:3. The amount of arginine was 40 times higher in Swiss Emmentaler. These differences may be due to different manufacturing processes and different strains of starters used.

The sweet taste attributed to proline in Swiss cheese may also be due to the group of sweet amino acids: glycine, alanine, proline, serine, and threonine, but the proline content is much higher than any of the other sweet amino acids. The ratio of bitter amino acids to sweet amino acids was 2.33 in poor-quality Swiss cheese, but in high-grade Swiss cheese, it was 1.93 to 2.05 in studies by Dylanyan et al. (99), Schormüller et al. (379) found that the amino-acid composition of the tear fluid of Swiss cheese was qualitatively, but not quantitatively, equal to that of casein. Proline content was about twice as great in Swiss cheese had an "alpha-ripening," which means that the greatest change took place around the a-casein peak, but cheeses such as Port Salut and Camembert, showed a "beta-ripening." Instances of nonspecific casein degradation,
as in Tilsit and Limburg, also were found. During ripening of Svecia cheese, Lindqvist and Storgårds (255) found that the first stage was decomposition of a component of the α-casein peak, probably k-casein. Approximately at the same time, degradation of the β-casein takes place. α-casein is not attacked until after a longer period of ripening. The same changes were observed with Swiss cheese (325) but Dyachenko et al. (99) do not believe that this division of the ripening process into different categories is justified.

This degradation of caseins could be caused by rennet, the natural proteinase of milk, and microbial proteinases but Stadhouders (404) found that the proteolytic activity of rennet and the milk enzyme was of only minor importance during cheese ripening and that the main enzymes were microbial proteinases. Poznanski and Rymaszewski (387) studied the proteolytic activity of S. thermophilus and L. bulgaricus in Edam cheese ripening. Addition of L. bulgaricus gave a satisfactory flavor, better than S. thermophilus, although addition of Micrococcus caseolyticus gave a bitter cheese (337). Only the endoenzymes of L. bulgaricus acted appreciably on whole casein, and those from S. thermophilus and M. caseolyticus had greater effects on intermediate products from casein. The activity of the bacterial enzymes was found to be low compared with the activity of rennin, and the effect of combined enzymes was greater on α-casein and k-casein than on whole casein (336). The enzymes produced by S. thermophilus and L. helveticus decomposed, to a varying extent, para-casein degradation products formed as a result of rennet hydrolysis (335). Proteinases of lactic-acid bacteria seem to play a particular role in flavor development. Rapp (342) observed that L. acidophilus showed the highest proteolytic activity followed by L. bulgaricus, L. helveticus, and L. casei, in that order. Dyachenko et al. (96) observed that 11 thermophilic Lactobacillus strains showed higher activity than 3 S. thermophilus strains. Lactobacillus helveticus showed the highest activity. Since strain differences within species can sometimes be greater than between representatives of different species, ratings of this nature are risky at best. Studies by Ohmiya and Sato (301) indicated that the extent of casein hydrolysis by intracellular proteases of S. cremoris and L. helveticus is similar to that of rennin. Aseptic rennet curd and aseptically harvested and washed cells of S. cremoris and L. helveticus were sandwiched between aseptic rennet curd. The curd was then coated with wax and incubated. Rapid autolysis of the cells occurred (302). The nonprotein nitrogen content increased, even in the aseptic curd during ripening at 10 C (50 F), but the increase was much smaller than in aseptic curd inoculated with S. cremoris and L. helveticus (303). This indicates that rennet is more active than Stadhouders (404) assumed. α-casein was most easily degraded by the lactic acid bacteria. On the contrary, Dyachenko and Shilovskaya (97) observed that L. bulgaricus, L. helveticus, S. cremoris, and S. thermophilus decomposed β-casein more rapidly than α-casein. The methods used to measure proteolytic activity also are important. Lactobacillus thermophilus showed much greater activity when assayed by measurement of tryptophan and tyrosine than by formol titration, although the reverse was true for S. thermophilus (300). Proteolytic enzymes have not been found in propionibacteria (28, 207).

The products of the proteolysis of the caseins are peptides. Schormüller and Belitz (377) showed that during the first 14 days of the ripening of Harz cheese that the amino acid content increased and then decreased to the 21st day. The amounts of peptides increase progressively during this period. In all cheeses investigated a characteristic peptide typical for casein degradation was isolated. Using electrophoresis, Storgårds and Lindqvist (415) investigated the peptide distribution of Svecia, Herrgard, Swiss, Port Salut, Blue, and Camembert cheese and found that all had a characteristic peptide composition. By more detailed studies it may be possible to isolate the different peptides and obtain a more exact picture of the differences in proteolysis between different cheeses.

The best known flavor influence of peptides is, of course, that they may produce a bitter flavor, which is considered a defect in Swiss cheese. But usually during ripening, the peptide content decreases (415). Since many of these peptides are phosphopeptides, which are not attacked by peptidases, phosphatases must remove the phosphates before the peptide can be degraded to amino acids. Phosphatases have been found in cheese, but because of their low activity in ripening cheeses, they may be the limiting factor for peptide degradation (376). Peptidases are found in many microorganisms, but they have not been studied as much as the proteinases (376). Bipeptidases, amino peptidases, and carboxypeptidases were found in sour-milk cheese (383), which showed the same pH-maximum as peptidases of microbial origin. The peptidases in the cheese were quite similar to the enzymes found in yeasts, bacteria, and animal tissues (376). Peptidases also have been found in propionibacteria, and these enzymes have an optimum pH close to 5.5 to 6.0 (28), which may be of importance in Swiss cheese. Schormüller and Müller (380, 381) were especially interested in the enzymes prolinase and prolidase in sour-milk cheese because free proline has always
been found in cheese. Neither prolinase nor proli-
dase are found in the cheese or in the fresh curd, but
they develop during ripening from the outside to the
inside. Their highest activity in sour-milk cheese was
found close to the rind, and the activity decreased
toward the core. Prolidase activity also has been
found in Swiss cheese (381).

The peptides formed during casein decomposition
may produce a bitter taste in the cheese if certain
microorganisms are deficient in the peptidases that
participate in peptide degradation (376). Stadhou-
ders (404) found that bitter cheese most frequently
developed at pH 5.2. The starters that decompose bitter
polypeptides may differ entirely in characteristics
from the nondecomposing starters. This capability
was specifically marked for some strains, and it was
not connected with the capability to liberate free
amino acids.

This may be because a pyrrolidone carboxylic acid
at the N-terminal end of a hydrophobic peptide
derived from casein may cause the bitterness in some
instances (108, 420). It was suggested by Sullivan
and Jago (420) that removal of bitterness by non-
bitter starters was due to the presence of a pyrroli-
done carboxyl peptide; a view that was opposed by
Exterkate and Stadhouders (108), who found this
enzyme in higher amounts in bitter than in non-bitter
strains of S. cremoris.

The amino acids produced by this process will be
more or less decomposed or rebuilt by microorganisms
in the cheese. Changes in the amino acid spectrum of
milk induced by the growth of two strains of S.
thermophilus and four strains of Lactobacillus
have been recorded by Grudzinskaia and Koroleva
(140). Amino acids present in considerable amounts in milk
were glutamic acid, glycine, threonine, alanine, tyro-
sine, methionine, and valine. Streptococci removed
almost all of these amino acids during their early stages in milk. Growth of L. bulgaricus and L. acid-
ophilus left the glutamic content almost unchanged,
reduced that of threonine, and led to accumulation
of histidine, arginine, tyrosine, phenylalanine, leucine,
cysteine, methionine, and valine. Streptococci intensified both utilization of amino acids present and formation of others. It has been observed by Kiuru et al. (204) that, during eye formation in Swiss cheese, the quantity of certain amino acids either is constant or declines. After 90 days, some amino acids increase greatly, but others decrease. Free aspartic acid disappears probably because of aspartase, which has been found in propionibacteria (451). After 45 days, the arginine content also is close to zero. Alanine, valine, serine, tyrosine, aspartic acid, glutamic acid, arginine, cystine, and methionine are degraded by propionibacteria (9).

The most important degradation products from
amino acids with reference to flavor are probably
amines, which are produced by decarboxylation of
amino acids. The following amines have been found
in Swiss cheese: tyramine (213), histamine (440), γ-
aminobutyric acid and cadaverine (39), and putrescine
(426). The hard cheeses seem generally to have low
amounts of these amines (376). The amino-acid decar-
boxylases occur only sporadically in lactobacilli
(376), and Dacre (68) found only one strain of Lacto-
bacillus with a high amount of tyrosine decarboxy-
late. Amino acid decarboxylase activity has not been
reported for propionibacteria. The tyramine content
found in cheese has been attributed to Kosikowski
and Dahlberg (213) to tyrosine decarboxylase produc-
ed by streptococci, especially S. faecalis. Other dis-
advantageous bacteria, such as coliforms, also may
produce these compounds (376).

Serine and threonine may be decomposed to α-
amino butyric acid and α-alanine in sour-milk cheese
according to Schormüller (376). Arginine, which has a repulsive, bitter, sweet taste, was shown by
Schormüller and Tänzler (382), to be decomposed via citrulline and ornithine to putrescine by arginine
desimidase and is, therefore, eliminated as a flavor-
diminishing amino acid. Transamination reactions
also take place during the metabolism of amino
acids.

Sulfur-containing compounds

These compounds must usually originate from the
sulfur-containing amino acids: cysteine, cystine, and
methionine. Formation of hydrogen sulfide has been
investigated most intensively. Few microorganisms
seem to deaminate sulfur-containing amino acids,
but biological hydrogen sulfide formation from cyste-
ine and cystine has been proven for Escherichia
coli (61). Similar possibilities also have been obser-
vated for methionine (127). Desnuelle and Woolley
(83) noted that propionibacteria liberated hydrogen
sulfide rapidly from cysteine. Lactic acid activated
this liberation. Desulphhydrase activity has been ob-
served in Cheddar cheese (55). It is understandable,
therefore, that hydrogen sulfide always occurs in
cheese—although in small amounts. Kristoffersen
(220) and Kristoffersen et al. (225) showed that concen-
trations of hydrogen sulfide and fatty acids were
related to the flavor of Cheddar cheese although
Kristoffersen (221) also observed sulfhydryl groups
in Swiss cheese and found that they were not related
to the cheese quality. The occurrence of active sul-
hydryl groups, however, seems to coincide with the
onset of eye formation (222). Redox-potential and
copper content seemed to have no effect on forma-
tion of active sulfhydryl groups, but the rate of acid
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development affected the concentration of sulfhydryl groups.

Besides hydrogen sulfide, mercaptans, thioethers, and dialkyl-disulfide have been found in cheese (376). Studies by Purvis et al. (341) with 35S-labelled milk showed that radioactive sulfur compounds were formed in Cheddar cheese. Methyl mercaptan was isolated from Cheddar cheese by Libbey and Day (251). The amounts of mercaptans, thioethers, and dialkyl-disulfides were found in larger amounts in Cheddar cheese made from raw milk than from pasteurized milk (224). Methyl mercaptans were found to be important flavor compounds in soft cheeses, as Limburger and Romadur (180), and also in semisoft cheeses, as Port Salut and Trappist cheese (138, 139), but they seem of little importance in Swiss cheese. But, sometimes, an undesirable influence of sulfur compounds on the taste and odor of Swiss cheese may occur. A German patent by Karrer (182) attempts to eliminate this off-flavor (which may be due to formation of sulfur compounds during the in vat treatment in steel vats) by suspending a copper plate in the vat. An electrolytic effect is produced, and the sulfur compounds are deposited on the plate.

The most important sulfur-containing compound in Swiss cheese is dimethyl sulfide (241), which may be an important contributor to Swiss cheese flavor. Dimethyl sulfide was found in amounts from 0.056 to 0.183 mg/kg in Swiss cheese (241, 244). Dimethyl sulfide was found to be a normal constituent of milk and was found to contribute to the milk flavor (324, 343). The amount of dimethyl sulfide found in Cheddar cheese was much lower than in Swiss cheese and was expected to have no influence on flavor (241). The flavor threshold in bland milk fat was 0.024 mg/kg, and dimethyl sulfide constitutes the fresh bouquet of butter (76). The concentration of dimethyl sulfide was about four times this value in Swiss cheese (241). Part of the dimethyl sulfide content in Swiss cheese may be produced by chemical degradation of methional (72), but it is generally believed that this compound is produced by microbial metabolism. A strain of Enterobacter aerogenes, which caused a cowy flavor in milk due to dimethyl sulfide, was found to produce dimethyl sulfide in amounts that caused this flavor (434). In a study of the production of volatile compounds by P. shermanii, Keenan and Bills (183) found a strain that produced dimethyl sulfide in quantities significant from the standpoint of flavor. This strain was found by Dykstra et al. (98) to produce dimethyl sulfide at both 8 C (46.4 F) and 30 C (86 F). Methionine, cysteine, cystathione, or β-dimethylpropioethin failed to enhance dimethyl sulfide accumulation. A cheese-whey fraction enhanced dimethyl sulfide production, as did precipitated whey protein or α-lactalbumin. Dimethyl sulfide, in this instance, is produced from sulfur-containing amino acids in peptide linkage.