SYMPOSIUM ON MICROBIALLY INDUCED FLAVORS AND FERMENTED FOODS

Introduction

As an introduction to this Symposium it is appropriate to reflect that microbially induced flavors and fermented foods played a most significant role in the founding of the science of microbiology.

The commercial value of wine led the great chemist and experimentalist, Louis Pasteur, to discover the fermentative powers of yeast and bacteria. He was the first to demonstrate that certain microorganisms are economically significant because of their ability to produce flavor compounds. He was the first to demonstrate that flavor of a fermented beverage could be improved and controlled by a heat process, which bears his name, because he understood the powers and properties of both desirable and undesirable microorganisms.

Today we can more completely appreciate the powers of a great many microorganisms, but we are still quite unable to explain many of the things they can do. We are indebted to Pasteur for launching the study of their activities, but we are indebted to microorganisms for continuing to give us a variety of many wonderfully flavored foods and beverages such as yogurt, cheese, bread, wine, beer, etc.

The chemical changes induced by microorganisms in these foods are varied and complex. Most are a result of the organism's attempt to sustain life in the food, but some occur after the organism's death, when its intra-cellular enzymes are released for activity. In many instances, it is not possible to duplicate a microbially induced flavor because more research is needed to identify the chemical constituents elaborated by microorganisms and their enzymes. Moreover, some of the already identified flavoring constituents can only be produced economically by a microbial process. Thus, the production of the L-isomer of glutamate and of the 5′-nucleotides relies almost completely on microorganisms for commercial production. Other complex flavoring materials will undoubtedly have to await biosynthetic or controlled biodegradative methods before becoming commercially available.

In organizing this Symposium, an attempt was made to encompass most of the important foods in which microbially induced flavors occur. Unfortunately, the subject of vegetable fermentations such as pickles, sauerkraut, and olives is omitted, but I am pleased by the representation in the areas of dairy, sausage, fish, bread, and oriental food products.

I trust that the Symposium will inspire interest and research toward an improved understanding of flavor production by microorganisms and their enzymes.

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Microbially Induced Flavors and Fermented Foods

Flavor in Fermented Dairy Products

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Compounds believed involved in flavor and aroma of nonripened fermented dairy products include lactic and acetic acids, acetaldehyde, formic acid, ethanol, carbon dioxide, diacetyl, dimethyl sulfide and other sulfur compounds, methyl ketones, primary and secondary alcohols, methyl, and ethyl esters of aliphatic acids and lactones. Homofermentative lactic streptococci (*Streptococcus lactis* and *Streptococcus cremoris*) ferment milk lactose by the hexose diphosphate pathway to produce lactic acid with small amounts of the other products. Heterofermentative streptococci (*Streptococcus diacetilactis* and *Leuconostoc citrovorum*) use the hexose monophosphate pathway producing less acid and more of the other end products, depending on aeration; they also ferment citric acid, producing diacetyl via both α-acetolacetate and acetyl CoA. Over 100 compounds have been identified in the volatile fraction of Cheddar cheese; those believed important include acetic, butyric, caproic, and caprylic acids, hydrogen sulfide, glutamic acid, methional, and carbonyl compounds.

The capacity of microorganisms to convert the carbohydrates, proteins, and fats occurring naturally in foods to a large number of different chemical compounds allows man to introduce great variety into his eating and drinking experience. Cheese alone provides well over 1000 products, each different as a consequence of controlling the environment of the ripening curd such that certain organisms are favored in growth to flavor the finished product. This paper will be concerned with flavor in fermented dairy foods and three products have been selected—yogurt and cultured buttermilk as examples of nonripened fermented foods, and Cheddar cheese as an example of a ripened dairy product. Compounds believed to be important in the flavor

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Table I. Stimulation of *S. thermophilus* by Seitz Filtrates (10%) from Milk Cultures of *L. bulgaricus*[a]

Hr Incubation of *L. bulgaricus*	Acidity of *S. thermophilus*[b]
0	3.9
2	4.0
4	4.5
6	5.1
12	5.4
24	5.2

[a] Data from Bautista *et al.* (1966).
[b] Acidity expressed as ml of 0.1N NaOH to achieve pH 8.3 using a glass electrode.

Table II. Stimulation of *L. bulgaricus* in Skim Milk to Which was Added 30% Neutralized and Pasteurized Filtrate of 20-hr Milk Culture of *S. thermophilus* Grown under Different Conditions[a]

Growth conditions of *S. thermophilus*	4-hr *L. bulgaricus* acid[b] + *S. thermophilus*	4-hr *L. bulgaricus* acid[b] Control
Nitrogen	55	..
Air, not shaken	55	34
Air, shaken	38	..

[a] Data from Galesloot *et al.* (1968).
[b] Acidity expressed as the number of ml of 0.1N NaOH required for the neutralization of 100 ml of milk to the phenolphthalein endpoint (pH 8.3).

Table III. Effect of Sodium Formate and Culturing under Nitrogen on Acid Production by a Yogurt Culture in Skim Milk at 45° C[a]

Milk heat treatment	Formate added (ppm)	Atmosphere[b]	Acidity after 3 hr[c]
10 min, 90° C	0	A	37
	150	A	93
	0	N	94
	150	N	94
30 min, 100° C	0	A	89
	150	A	91
	0	N	94
	150	N	92

[a] Data from Galesloot *et al.* (1968).
[b] A = air, N = nitrogen.
[c] See footnote b, Table II.

and aroma of each of these products will be indicated, as well as how, when known, each may be produced by the microorganisms.

Yogurt. Throughout the United States yogurt is increasing in popularity, but very few studies on this product have been carried out in this country. The organisms used in manufacture of yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, both of which are essential for proper flavor and aroma. Each of these bacteria produces compounds or a compound which stimulates the growth of the other during the fermentation; the effect of *L. bulgaricus* on the streptococcus is seen in Table I. These findings made by Bautista *et al.* (1966) clearly indicate that growth of the rod alters the milk, such that greater acid production by the coccus results. Pette and Lolkema (1950a,b) and Bautista *et al.* (1966) have shown that amino acids essential for *S. thermophilus*, especially valine, histidine, and glycine, are liberated by the lactobacillus. The effect of the coccus on the rod as reported by Galesloot *et al.* (1968) is shown in Table II. Here it may be seen that milk previously incubated with *S. thermophilus* under nitrogen or in air without shaking stimulated acid production by *L. bulgaricus*. Veringa *et al.* (1968) have identified the stimulatory compound as formic acid; this compound stimulated yogurt

Table IV. Carbonyl Compounds Produced by *S. thermophilus*[a]

Strain	Ppm Carbonyl Found: Acetaldehyde	Acetone	Acetoin	Diacetyl
98	2.2	2.9	1.8	tr[b]
216	2.1	1.2	1.6	0
100	1.7	5.2	3.0	tr
204	2.2	2.7	1.5	0
202	2.3	1.9	2.6	tr

[a] Data from Bottazzi and Vescovo (1969).
[b] Tr = trace (<0.05 ppm).

Table V. Carbonyl Compounds Produced by *L. bulgaricus* Strains used in Yogurt[a]

Group	Ppm Carbonyl Found: Acetaldehyde	Acetone	Acetoin	Diacetyl	Flavor
A	1.43	3.24	tr	0	atypical
B	3.88	2.44	tr	0	weak
C	8.50	3.00	tr	0	good

[a] Data from Bottazzi and Vescovo (1969).

Table VI. Taxonomic Tests for Lactic Streptococci and *Leuconostoc*

Organism	Acid coag. 48 hr 30° C	Diacetyl produced in milk	Ammonia from arginine	Dextran from sucrose	Precipitan group N antiserum
S. lactis	+	−	+	−	+
S. cremoris	+	−	−	−	+
S. diacetilactis	+	+	+[a]	−	+
L. citrovorum	−	+[b]	−	−	−
L. dextranicum	−	+[b]	−	+	−

[a] Some strains do not produce ammonia from arginine.
[b] When grown in association with lactic streptococci or when the pH is lowered to around 4.5 by adding acid.

production of acid in air when the milk was heated for 10 min at 90° C (Table III). If the air was replaced by nitrogen or if the milk was steamed or sterilized, added formate was without effect. Thus, *S. thermophilus*, when cultured under nitrogen, produced formic acid which stimulated *L. bulgaricus;* this effect was not demonstrable in steamed or sterilized milk which contains formic acid as a consequence of heating.

In addition to lactic and acetic acid, acetaldehyde is especially important in the flavor of yogurt (Bottazzi and Dellaglio, 1967). Table IV shows carbonyl compounds produced by yogurt streptococci according to Bottazzi and Vescovo (1969). Diacetyl was not significant and the aldehyde values were fairly constant around 2 ppm. However, aldehyde production by *L. bulgaricus* was more variable and those strains having the greater capability in this regard produced the best flavored yogurt (Table V). Acetaldehyde is a normal product of pyruvate catabolism by bacteria, but also can be formed by streptococci from thymidine by thymidine phosphorylase, deoxyriboaldolase, and deoxyribomutase catalyzed sequential reactions (Netherlands Dairy Research Institute, 1968). Threonine also can be converted to acetaldehyde and glycine by *L. bulgaricus* by means of threonine aldolase.

Buttermilk. Table VI shows the species important in the manufacture of cultured buttermilk. It has been known for many years that the streptococci produce lactic acid from lactose while the *Leuconostoc* and *S. diacetilactis* produce diacetyl from citric acid, especially as the pH falls below 6.0 during the fermentation. There apparently are two mechanisms for diacetyl synthesis operating in citric acid fermenting bacteria used in making cultured buttermilk. One involves

the condensation of active acetaldehyde and acetyl CoA (Speckman and Collins, 1968) and the other an oxidative decarboxylation of α-acetolactic acid (Inoue *et al.*, 1968; Seitz *et al.*, 1963a; Swomadainen and Ronkeinen, 1968). A widely used method for determining diacetyl involves forced nitrogen distillation at 60° C followed by conversion to dimethyl glyoxime in the presence of hydroxylamine (Pack *et al.*, 1964). The apparatus is illustrated in Figure 1. This same equipment is used to measure the acetaldehyde content of cultures by reaction with 3-methyl-2-benzothiazolone hydrozone (Lindsay and Day, 1965). The balance between these two compounds is critical in cultured buttermilk, for if the diacetyl to acetaldehyde ratio falls below 3 to 1, the green flavor defect results (Lindsay *et al.*, 1965a).

The desirable level of diacetyl in cultured buttermilk when consumed is 1.0 to 2.0 ppm. Unfortunately, this is too infrequently attained because the streptoccoci, especially *S. diacetilactis*, destroy diacetyl as it is produced (Seitz *et al.*, 1963a,b), the level usually being reduced from the 5 to 8 ppm present at 6 hr to less than 0.5 ppm after 12 hr. The loss of diacetyl is brought about by diacetyl reductase in the following manner:

$$CH_3COCOCH_3 + NADH + H^+ \rightarrow CH_3COCHOHCH_3 + NAD^+$$

This is an interesting enzyme from several standpoints, one of which is its apparent irreversible nature; reduction of NAD^+ in the presence of acetoin does not occur in crude or purified preparations which readily catalyze the forward reaction. The next step in the reaction sequence, that of the reduction of acetoin to 2,3-butylene glycol, is, however, readily reversible (Seitz *et al.*, 1963a). Since the presence of diacetyl causes serious flavor defects in citrus juices, beer, and distilled liquors, advantage may be taken of the irreversibility of diacetyl reductase to eliminate buttermilk off-flavor in these products. The enzyme has an optimum pH of 7.0, which makes it unsuitable for use in these acid products with pH levels around 4.0. However, a method of protecting the enzyme and regenerating reduced NAD has been developed in our laboratories (Whinery, 1969) so that commercial use of the enzyme, especially in beer, seems likely.

Cultural methods have been developed to overcome the consequences of diacetyl reductase activity in lactic starter cultures (Elliker *et al.*, 1964; Pack *et al.*, 1966; Pack *et al.*, 1967). One of these involves the addition of a 6-hr *S. diacetilactis* culture to buttermilk when the curd is broken at cooling (Pack *et al.*, 1967). Such a culture also can be added to the cottage cheese dressing used to cream the curd. These young cultures provide the levels of diacetyl desired and the stability of the flavor is then assured in the cold product, provided contamination by psychrophilic spoilage bacteria, which also contain diacetyl reductase (Seitz *et al.*, 1963b), is not significant.

Another approach to stabilization of diacetyl flavor in cultured products has involved attempts to isolate mutants of *S. diacetilactis* lacking diacetyl reductase (Burrows *et al.*, 1970). We have several of these in our collection now which have been produced using the chemical mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Table VII shows properties of some of these mutants isolated from the parent strains 26-2 and 18-16. While they lack diacetyl reductase activity, they also are unable to produce diacetyl. In fact, since this table was prepared, even the low levels shown here no longer are produced by the mutants. These strains also have enhanced acid-producing properties and vary in acetaldehyde production from those producing more than the parent to those producing less. All of these mutants contain citrate permease and thus appear to be blocked beyond the pyruvate oxidation step. They are not suitable for use in fermentations because of the poor diacetyl to acetaldehyde ratio, but attempts to isolate useful strains in this manner are continuing.

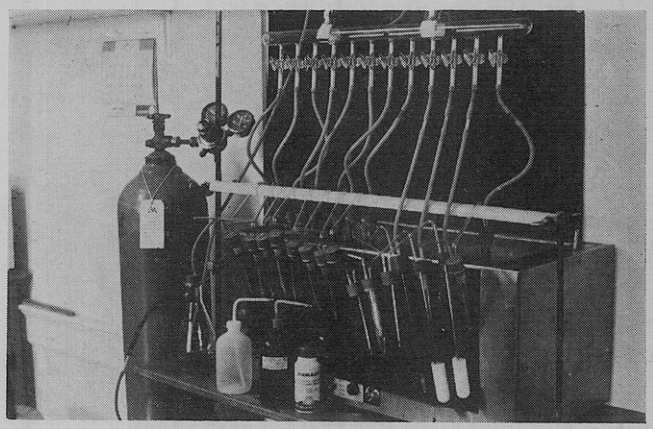

Figure 1. Apparatus used for diacetyl determinations, showing gassing manifold (top), reaction, and trapping vessels

Table VII. Properties of Mutants of *S. diacetilactis* Lacking Diacetyl Reductase[a]

Strain	Diacetyl (ppm)	Acetaldehyde (ppm)	Acidity (%)	Diacetyl Reductase (relative units)
26-2[b]	3.72	4.46	0.36	100
6	0.07	5.22	0.48	0
2 A	0.35	2.08	0.47	0.20
59	0.35	2.90	0.52	0.22
77	0.25	3.77	0.46	...
94	0.41	2.66	0.50	...
266	0.44	3.77	0.49	...
18–16[b]	2.41	3.64	0.22	65.7
3 N	0.19	3.12	0.33	0.08
4 N	0.25	1.06	0.32	0.16
5 N	0.35	1.03	0.32	...

[a] Data from Burrows *et al.* (1970).
[b] Strains 26–2 and 18–16 were wild type and the mutants derived from each are listed under the parent.

Table VIII. Acetic Acid and Ethanol Produced by *Leuconostoc* Strains Incubated in Nonfat Milk 48 hr at 30° C[a]

	Static Milk		Shaken Milk	
Strain	Acetate (μg/g)	Ethanol (μg/g)	Acetate (μg/g)	Ethanol (μg/g)
1–7	1240	485	2680	8
7–6	800	66	1150	3
32–7	1410	470	2710	8
91404	1180	755	2630	15

[a] Data from Keenan (1968).

The ability of *Leuconostoc* strains to reduce acetaldehyde to ethanol by means of alcohol dehydrogenase makes these organisms important contributors to proper balance of flavor in cultured buttermilk (Keenan *et al.*, 1966). Recent data from Keenan (1968) showing ethanol production at the expense of acetate in static 48-hr milk cultures is seen in Table VIII. Under aerobic conditions, acetyl phosphate is converted to acetic acid and ATP is formed during this reaction catalyzed by acetokinase. Under anaerobic conditions, this energy is unavailable to the cells, since they are compelled to reduce acetyl phosphate to acetaldehyde and the aldehyde to ethanol in order to oxidize the reduced pyridine nucleotides

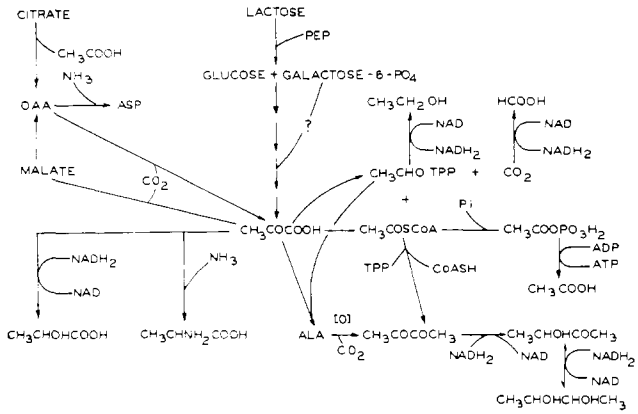

Figure 2. The possible fates of lactose, citrate, and pyruvate during carbon catabolism by lactic streptococci

needed to metabolize hexose by the hexose monophosphate pathway. In the presence of oxygen, these oxidized cofactors are undoubtedly provided through flavoprotein oxidases and peroxidases:

$$NADPH_2 + FAD \rightarrow FADH_2 + NADP$$

$$FADH_2 + O_2 \rightarrow FAD + H_2O_2$$

$$H_2O_2 + NADPH_2 \rightarrow 2H_2O + NADP$$

We recently examined lactic streptococci for the key enzymes of the hexose diphosphate (HDP) and hexose monophosphate (HMP) pathways, and at the same time followed the production of CO_2 from substrates labeled in various positions in order to determine how much of each of these metabolic routes is used in carbohydrate catabolism during milk fermentation (Nandan, 1967). Specific activity of the various enzymes for some of the strains are shown in Table IX. All strains contained aldolase and triosephosphate isomerase, indicative of the HDP pathway; noteworthy was that the *S. diacetilactis* strains, viewed by some as heterofermentative organisms, were as active as *S. lactis* and *S. cremoris* in this regard. All strains also revealed similar high levels of the enzymes alcohol dehydrogenase, acetokinase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic acid dehydrogenase, the latter two being key enzymes of the HMP pathway. Results of the radiorespirometric studies, however, revealed that the HDP pathway was quantitatively much more significant in carbon catabolism in these organisms than the HMP pathway; only 2 to 3% participation of the HMP or direct oxidative pathway was found. Thus these organisms are primarily committed to produce lactic acid from lactose and lesser amounts of other compounds are produced.

These and other important catabolic reactions in lactic streptococci are summarized in Figure 2. Pyruvic acid is a key intermediate and can be produced either from lactose or citrate by *S. diacetilactis* and *Leuconostoc* but only from lactose by *S. lactis* and *S. cremoris*. The pyruvate produced from lactose by the streptococci is apparently unavailable for diacetyl synthesis because of the tightly coupled reactions between glyceraldehyde-3-phosphate dehydrogenase and lactic dehydrogenase, which recycle oxidized and reduced pyridine nucleotide. So even though *S. lactis* and *S. cremoris* may have the capability of producing diacetyl from pyruvate, little or none is formed. *S. diacetilactis* and *Leuconostoc*, however, produce significant amounts of diacetyl from the pyruvate elaborated from citric acid.

Only recently was it shown that lactic streptococci do not ferment lactose like *Escherichia coli;* McKay *et al.* (1969) demonstrated that all three species of the lactic group convert lactose to glucose and galactose-6-phosphate by a phosphoenolpyruvate dependent reaction. Fate of the galactose derivative is under investigation.

Gas chromatography and mass spectrometry have been employed to identify over 50 volatile compounds present in cultured buttermilk (Lindsay, 1967; Lindsay *et al.*, 1965b). Most volatiles identified were also present in heated milk, and include aldehydes, methyl ketones, primary and secondary alcohols, methyl and ethyl esters of aliphatic acids, and sulfur compounds. Diacetyl and acetic acid, however, have been found only in cultured milk. From these findings, a synthetic culture flavoring mixture has been developed (Lindsay *et al.*, 1967) containing acetic acid, acetaldehyde, diacetyl, and dimethyl sulfide. When added in the proper amounts (30.0, 0.2, 1.0, and 0.025 ppm, respectively, in the finished product) these compounds will impart a typical buttermilk flavor to whole milk heated 1 hr in boiling water and then acidified with δ-gluconolactone.

Cheddar Cheese. Identification of compounds important in the typical flavor and aroma of Cheddar cheese has occupied research scientists for many years, but recent activity in this regard has been especially intense. A number of articles have reviewed the salient features of these studies, including those of Marth (1963), Mabbitt (1961), Day (1967), and Schormüller (1968). As these authors point out, a great many compounds are no doubt involved in the flavor and aroma of high quality Cheddar cheese. Indeed, over 100 compounds have been identified and these include acidic and neutral carbonyl compounds, nitrogen compounds, sulfur compounds, fatty acids, and miscellaneous compounds such as salt and fat. Figure 3 shows the separation of the taste and aroma compounds possible by vacuum distillation (Harper, 1959). Among the nitrogen compounds, the amino acids have been studied extensively, especially those present at different stages of ripen-

Table IX. Specific Activity of Various Enzymes in Cell-Free Extracts of Lactic Streptococci[a]

	Enzyme Measured (units/mg protein)					
Organism[b]	Aldolase	Triosephosphate isomerase	Alcohol dehydrogenase	Glucose-6-phosphate dehydrogenase	6-phospho-gluconic acid dehydrogenase	Acetokinase
Sd M8	1750	...	144	725	143	2261
Sd 18-16	1691	599	...	1099	89	...
Sc M1	1769	454	294	1580	...	525
Sc 144F	1987	...	89	919	119	1921
Sl E	...	...	129	...	52	1260
Sl C_2F	2461	363	...	1397	...	...

[a] Data from Nandan (1967).
[b] Sd = *S. diacetilactis*, Sc = *S. cremoris*, Sl = *S. lactis*.

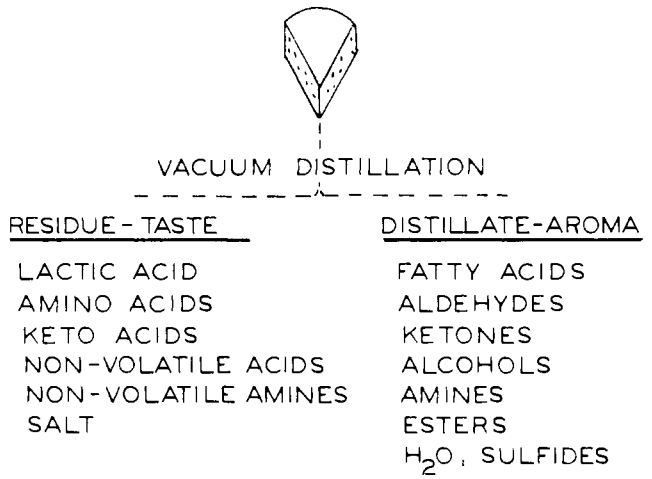

Figure 3. Separation of taste and aroma components of cheese flavor by vacuum distillation
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Table X. Average Fatty Acid Composition of Cheddar Cheese[a,b]

Acid	mg/kg	Acid	mg/kg
2:0	865	14:0	218
4:0	115	16:0	503
6:0	38	18:0	172
8:0	41	18:1	467
10:0	49	18:2	69
12:0	81	18:3	40

[a] Average of 14 samples. [b] Data from Bills and Day (1964).

ing (Kosikowski, 1951; Marth, 1963). No combination of amino acids has been found to produce Cheddar flavor, but they no doubt provide a background of flavor and thereby play an important role in the taste of the cheese. Various amines produced by decarboxylation of amino acids also have been isolated from normal Cheddar cheese (Day, 1967). These include tyramine, cadavarine, putrescine, and histamine. The Strecker degradation of various amino acids, especially methionine, to the corresponding aldehyde of one less carbon atom also contributes to cheese flavor. In fact, Keeney and Day (1957) found that methional produced in this manner had a characteristic cheese aroma. Amino acids may also be converted to aldehydes by transamination and decarboxylation reactions.

Fatty acids also are significant contributors to cheese aroma, and the average amount and type of each found by Bills and Day (1964) in normal Cheddar cheese are shown in Table X. All of the even-numbered carbon compounds were present up to C18, as well as C18 acids having one, two, and three double bonds. Until recently, little attention has been given to the lypolytic activity of bacteria which might be responsible for the liberation of these fatty acids in cheese. Workers in Japan (Sato *et al.*, 1967; Umemoto *et al.*, 1968) have demonstrated this activity in both starter and adventitious bacteria normally found in Cheddar cheese. Data of Sato *et al.* (1967) for whole cells are shown in Table XI. The fatty acids titrated were liberated from emulsions of butterfat. Cell free extracts also were active as reported by Umemoto *et al.* (1968) (Table XII). Free fatty acids of C_{10}, C_{12}, C_{14}, C_{16}, and C_{18} liberated by extracts of *Lactobacillus casei* and *Lactobacillus plantarum* were identified by these workers, using gas chromatography.

A significant contribution on the role of fatty acids in

Table XI. Increase in Titratable Acid Liberated by Cells of Various Lactic Acid Bacteria from Butterfat Emulsions after Incubation for 48 hr at 37° C[a]

Organism	ml N/50 NaOH/100 mg dry cells
S. cremoris	1.5
S. diacetilactis	1.7
L. casei	1.5
L. casei	1.6
L. plantarum	1.9
L. helveticus	1.0
M. varians	1.0
M. luteus	5.9

[a] Data from Sato *et al.* (1967).

Table XII. Increase in Titratable Acid Liberated by Cell Free Extracts of Various Lactic Acid Bacteria from Butterfat Emulsion after Incubation for 48 hr at 37° C[a]

Organism	ml N/50 NaOH/mg N
L. casei	1.8
L. plantarum	5.2
L. helveticus	2.7
S. diacetilactis	1.9

[a] Data from Umemoto *et al.* (1968).

Table XIII. Relationship Between Free Fatty Acids plus Acetic Acid in Determining the Flavor of Cheddar Cheese[a]

Cheese flavor	Free fatty acids + acetate (μmoles)	Moles free fatty acid/μmoles acetate
Fine, Cheddar flavor	12 to 28	0.55 to 1.0
Fermented, rancid fruity, unclean	>28	>1.0
Flat	<12	<0.55

[a] Data from Ohren and Tuckey (1969).

Table XIV. Relationship of Total Plate Count of Milk to the Level of Free Fatty Acids plus Acetic Acid Found in Cheddar Cheese Analyzed after 3 months[a]

SPC/ml		Free fatty acids + acetate (μmoles/gm)	
Raw	H_2O_2	Raw	H_2O_2
1T[b]	300	18	11
7T	150	21	17
20T	9T	20	18
20M	50T	45	24
21M	16M	59	53
32M	10M	80	26

[a] Data from Ohren and Tuckey (1969). [b] T = thousand; M = million.

Cheddar cheese flavor was published recently by Ohren and Tuckey (1969). They found that typical Cheddar flavor was related to the balance between free fatty acids and acetate (Table XIII). Not only was the total amount of these compounds important, as seen in the second column, but the ratio was also important, as seen in the third column. The microbial content of the milk also has been shown to influence the free fatty acid and acetic acid content of Cheddar cheese (Table XIV). Unsuitable cheese with more than 28 μmoles of these compounds per g resulted from raw milk with standard plate counts of 20 million per ml or more. Treatment of such milk with hydrogen peroxide to reduce the count provided better flavored cheese as a consequence of lowering the free fatty acid plus acetic acid levels. Also evident from this table is the fact that milk with too low a plate count may yield a flat cheese with insufficient levels of these compounds.

Table XV. Effect of Incubation of Raw Milk at 37° C on the Concentration of Active -SH Groups and Flavor of Cheddar Cheese after 2 and 8 months[a]

Hrs Raw Milk 37° C	SH/100 g	Cheddar flavor
	2 months	
0	4.7	v. sl.
5	5.5	slight
	8 months	
0	3.5	v. sl.
5	13.0	definite

[a] Data from Kristoffersen (1967).

The presence of hydrogen sulfide, dimethyl sulfide, and methyl mercaptan in Cheddar cheese has been reported by a number of workers (Day, 1967), and these compounds no doubt contribute to the overall flavor of the product. Also, active sulfhydryl groups are apparently important in Cheddar flavor, as the data of Kristoffersen (1967) reveal (Table XV). Cheese made from raw milk held for 0 and 5 hr at 37° C was examined at 2 and 8 months of age. The direct relationship between the active sulfhydryl level and Cheddar flavor is evident.

Adding to the knowledge of the complex nature of the flavor of Cheddar cheese has been the identification of acidic carbonyl compounds such as oxaloacetic, oxalosuccinic, glyoxylic, pyruvic, α keto-glutaric, α acetolactic, and α keto-isocaproic acids in cheese distillates. Neutral carbonyls found include acetaldehyde, acetone, butanone, 2-pentanone, 2-heptanone, 2-nonanone, *n*-decanal, 2-undecanone, *n*-dodecanal, 2-tridecanone, 2-pentadecanone, acetoin, diacetyl, formaldehyde, 3-methylbutanal, propanal, 3-methylthiopropanal, and butanal (Day, 1967; Marth, 1963). Various alcohols, esters, and lactones also have been identified in Cheddar cheese (Bills *et al.*, 1965; Day, 1967).

Journal articles that attribute direct roles to certain microorganisms in determining the proper flavor and aroma of Cheddar cheese (Day, 1967) are plentiful. These include reports dealing with the starter bacteria and with the adventitious microorganisms of the *Lactobacillus*, *Micrococcus*, and *Pediococcus* genera. No findings have come from these studies, however, providing manufacturing techniques using any combination of these bacteria which will provide fine-flavored cheese with any consistency. In fact, only recently (McGugan *et al.*, 1968; Reiter *et al.*, 1967) has information been provided establishing the direct contribution of starter bacteria to the typical flavor of Cheddar cheese. Cheese was made at the National Institute for Research in Dairying at Reading, England, under aseptic conditions which prevented the entry of nonstarter bacteria. Some cheese also was prepared using δ-gluconic acid lactone as the acidifying agent in place of starter bacteria. Cheese made with only starter bacteria had a mild though typical Cheddar flavor while cheese made with the lactone was devoid of cheese flavor (Reiter *et al.*, 1967). These cheeses were examined for volatiles at The Food Research Institute in Ottawa, Canada. Gas-liquid chromatography and mass spectrometry detected the same volatiles in starterless cheese having no Cheddar flavor as in cheese made with starter and having a characteristic Cheddar flavor. Methyl disulfide and dimethyl sulfide were the only compounds consistently detected in higher concentrations in cheese made with starter than in cheese made without starter (McGugan *et al.*, 1968).

From these findings it appears that starter bacteria, though weakly proteolytic and lypolytic, are able to produce fatty and amino acids, carbonyl, and sulfur compounds, the balance of which yield Cheddar flavor and aroma; enzymes released by cell lysis or held in dead cells would be expected to function throughout ripening.

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