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The tendency for certain single- and mixed-strain lactic starter cultures to develop a fruity and/or fermented off-flavor in ripening Cheddar cheese was traced to certain strains of Streptococcus lactis in the starter. A very intense fruity and/or fermented flavor developed in experimental cheeses when one particular S. lactis strain was used as a component microorganism in the starter culture. Contrary to previous implications, strains of Streptococcus diacetilactis did not produce a fruity and/or fermented flavor in experimental cheeses made with starter cultures containing these organisms. Mixed-strain cultures of Streptococcus cremoris were used to produce control cheeses, which did not develop the fruity and/or fermented flavor defect.

The starter culture and treatment of the cheese milk were the only variables used in the cheesemaking trials. Results obtained

indicate that the specific species and/or strain of lactic streptococci contained in the starter culture affect the flavor score and type of flavor in the resultant cheese, independent of the temperature of the heat treatment or hydrogen peroxide-catalase treatment of cheese milk.

There were no apparent correlations between "normal" and "defective" starter cultures for the level of proteolytic activity or production of acetaldehyde or diacetyl to the development of fruity and/or fermented off-flavor in cheeses. Existing procedures for the quantification of acetaldehyde, diacetyl and volatile esters were adapted and modified for determining the concentration of these compounds in cheese curd and ripened cheese. The method of slurry preparation, type of diluent and the pH were factors found to affect the recovery rate for acetaldehyde from cheese.

The relative flavor preference for typical experimental cheeses was determined with the aid of a flavor panel. A sample of fermented-unclean cheese received the lowest hedonic flavor score, whereas the flavor score of a slight fruity and/or fermented sample compared favorably with that of the reference sample.

Lactic Streptococci and the Fruity Flavor Defect of Cheddar Cheese

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INTRODUCTION

Too frequently high quality Cheddar cheese is not available in the domestic market. Frequently problems encountered in the manufacture and aging of the cheese result in definite flavor, body and texture defects. Consumers regularly complain about the lack of uniformity and consistency of American Cheddar cheese.

Within the last ten years the cheese industries of the United States, Canada, Australia, and New Zealand have been puzzled by the recurrence of a most distinct off-flavor. This off-flavor has been variously described as fruity and/or fermented, "apple-like", "pineapple-like", "pear-like" or as a "lactis" flavor. It is imperative that cheese which develops this off-flavor be marketed at an early age, despite the serious flavor criticism. Cheese possessing the defect tends to develop a pronounced "unclean" flavor and a "sawdust" or gritty texture upon ripening beyond ten months. At this point the cheese is generally considered unpalatable.

The observations of several Oregon cheesemakers and the results of preliminary cheesemaking trials by researchers at Oregon State University suggested that the fruity flavor defect could be traced to certain commercial starter cultures. Further

work indicated that several particular species or strains of lactic streptococci could be responsible.

The present investigation was undertaken to extend the knowledge on the probable causes leading to the abnormal flavor defect and possible methods of control. The probable relationship of several suspect species and strains of lactic streptococci was emphasized in the study. It was especially desirable to determine the possible contribution of several strains of Streptococcus diacetilactis and Streptococcus lactis to the development of this defect. In conjunction with this part of the investigation, several commercial mixed-strain cultures containing the aforementioned species were employed as cheese starters.

Another objective was to obtain information that could serve as criteria for the selection of starter strains that would ensure the production of high quality cheese. Procedures for the quantification of diacetyl, acetaldehyde, and volatile esters were modified and adapted for the analysis of cheese curd and ripened cheese.

REVIEW OF LITERATURE

Biochemistry of Cheddar Cheese Flavor

The flavor, body and texture of Cheddar cheese is dependent upon the nature and extent of fermentation and chemical processes which occur in the cheese during various stages of manufacture and ripening. The main variables which determine the flavor of the finished cheese are: (1) microorganisms and enzymes indigenous to the cheese milk, (2) lactic streptococci, adventitious microorganisms and enzymes incorporated through processing, (3) conditions and methods of manufacture and (4) conditions of curing. Hence, given a single lot of milk, it is possible to produce cheeses with varied flavor properties.

Much evidence accumulated within the last decade supports the supposition that the flavor of ripened Cheddar cheese is due to the presence of many different chemical compounds (45, 48, 110, 122, 126). Day and Libbey (48) by means of gas chromatographic and mass spectral analyses, separated the aroma fraction of Cheddar cheese into approximately 130 components. Most investigators working with cheese flavor believe that some of the most significant compounds must occur in certain ratios of concentration if the cheese is to have the desired Cheddar flavor (126). The balanced component theory for typical Cheddar cheese flavor has therefore evolved (77,

105, 138, 179). This theory has become well accepted since earlier workers were not successful in isolating a single compound with a distinct cheese-like character from Cheddar cheese.

Numerous experiments have been conducted in an attempt to simulate Cheddar cheese flavor by adding simple mixtures of compounds into fresh cheese. Baker and Nelson (12) incorporated several amino acids into cheese curd. Generally, the end results were inconsistent or inferior cheese was produced. Silverman and Kosikowski (179) obtained a slight Cheddar-like flavor by the addition of amino acids and free fatty acids to bland curd. Other chemical compounds that have been added to cheese curd or slurries are various methyl ketones and fatty acids (197, 198), and carbonyl compounds with short-chain fatty acids and 3-mercaptopropionic acid (45). These attempts to simulate Cheddar flavor have met with only limited success. Day, Bassette and Keeney (45) stated that certain non-volatile products of proteolysis and lipolysis in the cheese probably have a significant influence on the over-all flavor.

Marth (126) enumerated the following classes of compounds found in Cheddar cheese that can apparently affect cheese flavor: (1) carbonyl compounds, (2) fatty acids and their derivatives, (3) sulfur compounds, (4) nitrogen compounds and (5) miscellaneous compounds.

Carbonyl Compounds

The volatile carbonyl compounds isolated and identified from Cheddar cheese are usually grouped into acidic and neutral carbonyls. The acidic carbonyls consist of various keto acids, which possess both the carbonyl and carboxyl function on the same molecule. The neutral carbonyls do not have a carboxyl function and consist of compounds such as aldehydes and ketones.

Acidic Carbonyl Compounds. Many cheese researchers have isolated and identified acidic carbonyl compounds from various types of cheese. Oxalacetic, pyruvic, alpha-ketoglutaric, alpha-acetolactic and alpha-ketoisovaleric acids were found in Cheddar cheese by Bassett and Harper (14). Kristoffersen and Gould (109) confirmed the presence of all of the above compounds except alpha-ketoisovaleric acid. Oxalsuccinic, glyoxylic, and alpha-ketoisocaproic acids were also found in Cheddar cheese by these workers. The latter authors found no correlation between the quantities of pyruvic and alpha-ketoglutaric acids isolated from the cheeses and the age, grade or flavor of the cheeses.

Neutral Carbonyl Compounds. Bassett and Harper (14) were able to identify 11 neutral carbonyls in extracts from 82 samples of eight cheese varieties. Many of the neutral carbonyl compounds are apparent degradation products of acidic compounds via

decarboxylation of the corresponding beta-ketoacids. For each neutral carbonyl, a possible acidic precursor can usually be found in cheese in significant concentrations. A notable exception, however, is the mode of formation for butanone, since neither valeric nor beta-ketovaleric acids occur to any extent in milkfat (85). Bills (15, p. 11) recently summarized the various neutral carbonyls that have been isolated from Cheddar cheese.

Because of its contribution to the aroma, diacetyl is one of the more significant neutral carbonyls in cultured dairy products (64, p. 17). Calbert and Price (24) examined 28 lots of Cheddar cheese and observed that the diacetyl content ranged from 0.2 to 3.55 parts per million (ppm). Less than 0.5 ppm of diacetyl was contained in 78 percent of the cheeses considered to have no flavor defects. All of the lower grade cheeses contained more than 0.5 ppm of diacetyl. These co-workers concluded that a small quantity of diacetyl was necessary for typical Cheddar flavor and aroma, but above a certain concentration level, this carbonyl could be responsible for cheese off-flavor.

Harvey and Walker (83) studied the development of carbonyl compounds in Cheddar cheese. In one-day-old cheese they found acetaldehyde, acetone, butanone, and 2-pentanone. After two to four weeks of curing they also found 2-heptanone. Also isolated and identified were 2-nonanone (20 to 24 weeks) and 2-undecanone

(36 weeks). These authors observed that the flavor of mature Cheddar cheese was first apparent after eight to 12 weeks and that the flavor becomes more pronounced as the concentration of 2-pentanone, 2-heptanone and 2-nonanone increased.

Scarpellino and Kosikowski (167) reported that butanone might be the most predominant carbonyl in Cheddar cheese, whereas Day and Keeney (47) indicated that 3-methylthiopropanal is a key component.

One of the conclusions from the work of Kristoffersen and Gould (109) was that no apparent general relationship existed between flavor and the amount of individual carbonyl compounds present in the cheeses that were analyzed. They also noted that if air entered the cheese prior to final curing, flavor was impaired and pyruvic acid concentration increased, but the level of alpha-ketoglutaric acid decreased and oxalacetic acid disappeared entirely.

Several scientists have established that a portion of the carbonyl compounds in ripened cheese were derived from casein. Wolin and Kosikowski (204) used tritium-labeled casein in Cheddar cheese-making to establish this fact.

Marth (126) cautioned that some of the work reporting the presence of carbonyl compounds in Cheddar cheese, as determined by means of steam distillation at atmospheric pressure, should be carefully interpreted. This precaution was expressed in light of

work by Lawrence (115), who found that extraction procedures involving the heating of dairy products (that contain milkfat) at atmospheric pressures can produce methyl ketones as artifacts.

Free Fatty Acids

An early report on the presence of fatty acids in cheese was that of Suzuki, Hastings and Hart (186) in 1910. These authors observed that: (1) volatile fatty acid levels increased throughout the ripening period, (2) formic acid could not be detected until the cheese was more than five months old, (3) acetic and propionic acids did not appear until all the lactose was fermented and maximum concentrations of these acids were reached at three months, (4) butyric and caproic acid levels continually increased, (5) valeric acid was not present and (6) lactates were the principal sources of acetic and propionic acids.

Bills and Day (17) employed several chromatography techniques to determine the predominant free fatty acids in 14 samples of Cheddar cheese. Formic and propionic acids were not found in any of the cheeses when determinations for fatty acids were conducted. Acetic acid was the most predominant free fatty acid and showed the greatest variability in concentration.

Petersen and Johnson (158) noted that most of the acetic and butyric acid originally present in the cheese milk were lost in the

whey. However, significant concentrations of these acids appeared again in the cheese within one day. These workers observed the presence of caproic, caprylic and capric acids in cheese after 30 days of aging and that their aroma was characteristic of Cheddar cheese.

Several investigators have shown evidence for fat hydrolysis in ripening Cheddar cheese (175). Lipolysis in Cheddar cheese has been comprehensively reviewed by Sjøstrom (181). Kannan and Basu (98) noted lipase activity in Cheddar cheese after 30 to 50 days of ripening, and a decline in this activity after 110 days.

It has been observed that no typical flavor develops in "Cheddar cheese" made from skimmilk (124). This immediately suggests that milk fat is an essential substrate for typical Cheddar cheese flavor development. Characteristic Cheddar flavor and aroma is generally recognized to be concentrated in the fat phase of the cheese and soluble in fat solvents (11, 36, 124). Patton (149), on the basis of flavor panel responses, concluded that acetic, butyric, caproic, and caprylic acids "constitute the backbone of Cheddar aroma". A study by Kristoffersen and Gould (109) related the ratio of free fatty acids and hydrogen sulfide concentrations to typical cheese flavor. Mattick and Hiscox (127) concluded that a high volatile acid content could be associated with the presence of high numbers of non-lactic bacteria. This conclusion was based on a study of the

relationship of fatty acid level in cheese to its microflora. It is known that fat hydrolysis generally proceeds more rapidly in raw milk than pasteurized milk cheese. Studies by Babel and Hammer (8), Irvine, Bullock and Sproule (91) and Peters and Williams (156) have demonstrated the possibilities of flavor improvement by inducing lipolysis by the addition of enzymes, Geotrichum candidum, or by homogenization of the cheese milk. Excessive lipolysis usually causes objectionable rancidity and/or bitterness (142, p. 211) in Cheddar cheese.

A portion of the free fatty acids partially responsible for Cheddar cheese flavor may arise through activity of lipase indigenous to milk (if it is not destroyed by pasteurization), microbial lipases, or lipases from other sources introduced into milk at the time of cheesemaking. The milk lipase active at an alkaline pH (8.5 to 9.0) would generally be inactive in Cheddar cheese (94, p. 195; 159). The inherent pH of Cheddar cheese would favor activity of the lipase system active at pH 5.0 to 6.0, as observed by Albrecht and Jaynes (2). Peterson, Johnson and Price (159) observed that milk lipase is inactive at the pH of Cheddar cheese and is not detected in the cheese after pressing. The latter authors also found that the bulk of the so-called "cheese lipase", active at pH 5, was produced between the fifth and hundredth day of curing.

Most lipases are inactivated by heat treatments less drastic

than pasteurization (94, p. 196). Data presented by Jenness and Patton (94, p. 201) indicates that a holding time of nearly four minutes is required to inactivate lipase at a temperature of 64° C. The prevalence of flash heat treatments (64° C for 15 seconds) of Cheddar cheese milk in the Midwestern United States places additional significance on the relative heat tolerance of milk and microbial lipases. Interestingly, non-lipolytic microorganism such as lactobacillus species have been reported to demonstrate lipolytic properties after autolysis (157).

Since the lipases indigenous to milk are closely associated with casein and the fat globule membrane (65; 94, p. 197), there is a concentrating process during cheesemaking. In a cheese system it is quite possible to have a number of simultaneous lipolytic actions, each with a different pH optima and varied specificity with regard to substrate and ester bond (94, p. 193-196).

Sulfur Compounds

Due to the extreme volatility and potent odor of many of the sulfur compounds, their role in the flavor of Cheddar cheese has received considerable attention. The thresholds of odor detection are very low for sulfur compounds such as ethyl mercaptan (2.0 parts per billion (ppb) (92), methyl mercaptan (2.0 ppb in water) (46), and hydrogen sulfide (1.1 ppb) (128, p. 52).

The presence of hydrogen sulfide in Cheddar cheese has been observed by several investigators (108, 109, 110, 196) and methods of quantification have been developed by Lawrence (116), Kristoffersen and Nelson (111) and Badings and van der Pol (10). A distinct correlation between the level of free fatty acids, concentration of hydrogen sulfide and flavor scores of 14 lots of 12-month-old Cheddar cheese was found by Kristoffersen and Gould (110). Their results were reported on the basis of a ratio of free fatty acids to hydrogen sulfide concentration. In a subsequent study by the same investigators (109), it was reported that there were distinct differences in the levels of free fatty acids and hydrogen sulfide between raw and pasteurized milk cheese. Generally, higher levels of this sulfur compound were detected in raw milk cheese. Walker (196), by means of a gas entrainment and steam distillation method, observed hydrogen sulfide as the only volatile sulfur compound in New Zealand Cheddar cheese.

Libbey and Day (118) substantiated the presence of methyl mercaptan (concentration estimated to be 3 to 30 ppb) in Cheddar cheese by gas liquid chromatography (GLC), thin-layer chromatography and ultraviolet spectral techniques. Patton, Wong and Forss (151) isolated dimethyl sulfide from Cheddar cheese.

The production of methional from methionine in Cheddar cheese has been studied by several workers (92, 100). Jackson (92) observed that this compound has a cheese-like odor at lower

concentrations. Bills (15, p. 13) reported a similar characterization of the aroma of dilute preparations of methional.

It has been suggested by several workers (76, 170) that hydrogen sulfide is a product of the metabolism of lactobacilli. This sulfur compound could presumably be formed by the breakdown of the amino acids cysteine, cystine, cysteic acid or methionine. Mabbitt (122) has suggested that the enzymatic approach of Schormüller and Tänzler (168) could be employed in this area of study. Kristoffersen (106) recently proposed that the formation of active sulfhydryl groups in Cheddar cheese during curing may be the key to the development of characteristic Cheddar flavor.

Nitrogen Compounds

The patterns of occurrence of amino acids in ripening Cheddar cheese has received much attention from numerous workers. The breakdown or hydrolysis of casein in Cheddar cheese plays a key role in the ripening process. All of the amino acids common to casein, as reported by Gorden and co-workers (71, 72, 73), have been isolated from ripened Cheddar cheese. Bills (15, p. 7) listed 19 amino acids that have been isolated and identified by various investigators. Amino acid studies have shown the presence in Cheddar cheese, several amino acids not found in casein and the classical whey protein fractions. Ornithine, for example, occurs

in cheeses; it probably is synthesized by the microflora of the cheese (123). Dacre (33) reported that the pattern of amino acids in Cheddar cheese differs qualitatively from casein, and hence indicates that various amino acids are liberated at different rates and that some may be further metabolized by the cheese microflora.

The relationship of amino acids to Cheddar cheese flavor was extensively investigated by early researchers (102), and the contribution of amino acids to Cheddar cheese flavor is now generally recognized. The following amino acid or casein-type systems all tend to have a broth-like flavor; (1) mixtures of amino acids added to Cheddar cheese (179), (2) peptide-amino fractions isolated from Cheddar cheese by ion exchange techniques (123) and (3) Cheddar cheese made from skimmilk (124). Mulder (138) first offered the hypothesis in 1952 that amino acids impart a brothy background taste to cheese upon which the typical cheese flavor is superimposed.

Amino acids would not be expected to contribute to cheese aroma, since they lack volatility. However, amino acids can serve as precursors for many volatile compounds which can contribute to Cheddar flavor. One example is the degradation of methionine to methional. Keeney and Day (100) proposed that a slow chemical interaction between amino acids and dicarbonyl compounds could produce flavorful aldehydes such as methional. Methional, as previously mentioned, possesses a definite cheese-like odor.

Various other amino acids are able to undergo Strecker degradation, oxidative deamination and decarboxylation to yield flavorful compounds. Several investigators (23, 102, 180) have concluded that tyramine is a decarboxylation product of tyrosine. Dahlberg and Kosikowski (39) suggested a possible relationship between the level of tyramine and the intensity of Cheddar flavor. However, they pointed out that tyramine itself is not responsible for Cheddar flavor. Tyramine was added directly to fresh cheese curd, but this resulted in no immediate Cheddar flavor character nor did it actually aid flavor development during ripening. Dacre (35) verified these findings in his studies.

Silverman and Kosikowski (177, 178) reported the presence of cadaverine, which is the decarboxylation product of lysine. Putresine, according to a report by Sharpe and Franklin (170), is produced from the well-known decarboxylation and hydrolysis of urea from arginine. The above potent odorous amines have been isolated from Cheddar cheese, although their importance to Cheddar flavor has not been fully clarified.

Kristoffersen (109) found no definite relationship between the concentration of ammonia in Cheddar cheese and the intensity of typical Cheddar flavor. This author reported that the ammonia concentration in both raw and pasteurized milk cheese increased at similar rates throughout 12 months of ripening.

Other Flavor Components

Other components indigenous to Cheddar cheese undoubtedly contribute to flavor either directly, indirectly as the precursors of flavor compounds, or even possibly through such complex phenomena as flavor potentiation (113), synergistic effects (11) and additive interaction (49). It is very probable that many of the chemical compounds identified in Cheddar cheese contribute very little, if at all, to the flavor due to their presence in concentrations below their flavor threshold values (FTV) (150) and the nature of the solvent phase (148).

Ethanol and various secondary alcohols have been isolated from Cheddar cheese (18, 48, 117, 166). Most of the alcohols probably contribute little to Cheddar cheese flavor because their flavor thresholds are usually quite high. However, since many esters have low flavor threshold values (15, p. 13), the alcohols may play an important role in the formation of flavorful esters. Bills (15, p. 13) listed the various esters which have been isolated from Cheddar cheese by Day and Libbey (48), Suzuki et al. (186), McGugan and Howsam (130) and Bills et al. (19).

Several studies have shown that the sugars disappear from cheese very early in the ripening process (60), thus limiting their direct effect on cheese flavor. The concentration and distribution

of salt, water and unmodified fat and protein fractions would certainly be expected to influence the flavor of the cheese also. The intermediate products of proteolysis, though not volatile, are believed to contribute to the taste of Cheddar cheese. Several investigators have implicated peptides as the cause of a bitter taste in Cheddar cheese (55, 56, 57, 185). Bills (15, p. 14-15) proposed that lactic acid may contribute more to the acid taste of Cheddar cheese than any other single component. Personal observations of the author indicate that high acid (sour) taste is the most frequent flavor criticism of commercial Cheddar cheese. Examination of cheese make-sheet records indicates that the acid taste defect frequently develops as a result of high acid milk, accelerated manufacturing procedures, milling at high acidities or the use of excessive lactic starter culture.

Flavor Defects in Cheddar Cheese

Ordinary terminology is quite inadequate for the task of describing characteristic Cheddar flavor. Flavor scientists contend that only limited progress can be made until cheese flavor can be described or defined in terms of known chemical substances. Analytical methods in flavor chemistry are rapidly facilitating the chemical definition of Cheddar cheese flavor. Nelson and Trout (142, p. 207) state that "high quality Cheddar cheese should have a

characteristic 'Cheddar' flavor, which implies a clean, nutty and pleasantly sweet flavor blend."

Early investigators reported off-flavors and gassiness in Cheddar cheese prior to 1900. One of the earliest published reports proposed that a common cause was the presence of certain lactic acid producing bacteria, which were capable of producing hydrogen and carbon dioxide during lactose fermentation (164). Moore and Ward (133) and Marshall (125) reported that the occurrence of Bacillus coli communis organisms in cheese milk was the probable cause of gas and taint production in Cheddar cheese. Harrison (80) attributed the development of a mottled color defect, as well as an undesirable odor and taste in Cheddar cheese, to the liberation of gaseous products by yeasts and/or coliform bacteria, Allen (4) reported that yeasts, certain lactic acid bacteria, coliforms and spore forming anaerobes could readily produce gassiness in Cheddar cheese and other dairy products. Whitehead (200) published findings in 1930 which noted that the addition of bacilli of the colon group to cheese milk just prior to cheesemaking resulted in significant off-flavors in the finished cheese.

Bitterness

In the last decade several papers have been published that established a correlation between the extent of proteolytic activity

of a given starter culture and the tendency for bitterness to develop in the resultant cheese. Czulak and Shimmin (32) have demonstrated that a cell-free extract from a single-strain starter known to yield bitter cheese liberated lesser amounts of amino-nitrogen from sodium caseinate than did another "non-bitter" single-strain cheese starter. Emmons et al. (57) found that seven of eleven strains of Streptococcus cremoris used in cheesemaking trials caused bitterness in cheese. Nitrogen analysis of the cheeses demonstrated less hydrolysis of peptides (bitter-tasting compounds) in the bitter cheese samples than in the non-bitter cheeses. The same authors (55) later reported that the intensity of bitterness usually decreased as the proportion of non-bitter cheese producing strain or strains in the starter increased. It was also observed that the average chain length of the trichloroacetic acid-soluble peptides and amino acids were longer in the cheeses having the most intense bitter taste. Similar relationships between proteolytic activity of lactic starters and cheese bitterness were noted by Stadhouders (185).

A study was conducted by Yamamoto and Yoshitake (206) in which the proteolytic activity of cheese starter organisms was compared with the number and amount of free amino acids in cheeses made without starter. Paper chromatography of cheese extracts was employed as the primary analytical procedure. Fifteen amino acids appeared in the starter-made cheese after 30 days of ripening,

but only three amino acids were detected in the non-starter cheese after a similar ripening period, and only seven amino acids were isolated after seven months of ripening.

Kosikowski and Mocquot (104, p. 146) summarized the various agents which could be responsible for bitterness in cheese. They listed such bacteria as Lactobacillus casei, Streptococcus liquefaciens and Escherichia coli in addition to an excess of rennet or high acidity of the cheese milk.

High Acid (Sourness)

Probably the most frequently incurred flavor criticism of Cheddar cheese is the high acid or sour defect. Accelerated or excessive acid development in the manufacturing process, high cheese moisture content or an abnormal fermentation are common causes of this defect (201). This off-flavor is quite often very intense. Generally, a "quick", sour flavor sensation is noted, which soon disappears, leaving the mouth of the taster free of any other off-flavor or after-taste sensations. An acid flavor is frequently associated with a dull, faded, acid-cut color (142, p. 209). Bills (15, p. 14) proposed that lactic acid possibly contributes more to the acid taste of Cheddar cheese than any other single acid component. This author noted that the pKa of lactic acid is 3.86, which would indicate that it is nearly ten times more acidic than acetic

acid (pKa 4. 76). In another study this worker observed that lactic acid is usually the most abundant short-chain organic acid in Cheddar cheese at various stages of ripening (17).

Frequently, Cheddar cheese will simultaneously exhibit an acid and a bitter taste. This is perhaps significant in light of the observation by Bills (15, p. 14) that a commercially prepared lactide of lactic acid had a pronounced bitter taste. This suggests that bitter-tasting cheese could eventually develop from an initial high acid-flavored cheese, as the result of two molecules of excess lactic acid esterifying to form the lactide (48). Day and Libbey (48) found evidence for the presence of cis and trans isomers of the lactide of lactic acid in Cheddar cheese, based on gas chromatographic and mass spectral analyses. Personal observations of the author bear out the fact that many lots of Cheddar cheese manifest a sour flavor early in the ripening period which is followed by the development of a distinct bitter taste after about six months of curing. This bitter taste may sometimes mask the high acid flavor.

"Whey", "whey-taint" or "sour-whey" are terms used to describe various intensities of off-flavors associated with incomplete or abnormal expulsion of whey from cheese (142, p. 211). The taste reaction is relatively "quick" and "brief" and the sampler's mouth "cleans up" soon after expectorating the sample. The slight acid taste and aroma associated with whey taint is characteristic of

fermented whey. This flavor defect is usually caused by incomplete expulsion of the whey from the cheese during critical phases of the manufacturing operation. Quite frequently this flavor defect arises when acid development is significantly depressed or halted within the cheese curd during the manufacturing process.

The yeasty flavor defect of Cheddar cheese is readily identified by its sour, yeasty taste and peculiar, fragrant odor (142, p. 211). Yeast growth generally is the cause of the defect, and is usually evidenced by the presence of yeast or sweet curd holes.

Wilson and Reinbold (202, p. 54) state that it is frequently difficult to distinguish between fruity and/or fermented, yeasty and whey-taint flavors. Unfortunately, many experienced cheese graders fail to agree on precise terminology when defining the above mentioned flavor defects. The terms fruity and/or fermented, yeasty and whey-taint are loosely employed by many cheese flavor evaluators; hence this has inadvertently resulted in classifying these off-flavors into a single quasi-category (202, p. 54).

Fruity and/or Fermented

The fruity or fermented flavor defect of Cheddar cheese has been described as "pineapple-like", "apple-like", "pear-like" (63) or suggestive of a typical fruitstore odor (142, p. 210). The taste is sweet and the aroma resembles that of fermenting or overripe

fruit. Nelson and Trout (142, p. 210) noted that this flavor defect may be associated with high moisture, weak, pasty-bodied cheese. These authorities also observed that the defect generally becomes more intense as the cheese is aged. The above descriptions of the fruity or fermented flavor of cheese closely coincide with the observations of the author during the course of this investigation.

Irvine and Beach (89) reported results from the analyses of 2,300 samples of commercial Canadian cheese and attempted to correlate compostion values with flavor qualities. Generally, the higher quality cheese had lower pH values than defective lots of cheese. These investigators concluded that most of the undesirably "sweet" or "fruity-like" cheese had significantly higher pH values or lower titratable acidities. The non-lactic bacterial counts of the poor flavor quality cheese were usually significantly higher than 20 samples of 94.0 score cheese. Cheese grading was based on the American Dairy Science Association score card, which allows a 95.0 score for no flavor, body and texture criticisms (192, p. 271). Irvine, Beach and Burnett (90) found that lower temperature heat treatments (128° and 133° F) for cheese milk resulted in the development of a slight fruity flavor in experimental cheeses.

Tuckey, Nelson and Hussong (191) encountered "old cream" and "fermented" flavored Cheddar cheese when the cheese milk was inoculated with Oospora lactis and S. lactis. A distinctive fermented

flavor persisted for about four months.

More recently, single strain cultures of S. lactis and S. cremoris were used by Perry (153) for the manufacture of Cheddar cheese. Three strains of S. cremoris produced good flavored cheese, whereas three strains of S. lactis yielded an abnormal fruity, "dirty" or "lactis" flavor as Perry described it. He noted that the characteristic flavor became more pronounced as the cheese ripened. Bacterial population studies were conducted in the course of the investigation. Perry found that the S. lactis strains seemed to survive at higher rates in the cheese than did the S. cremoris strains. After three weeks aging, the average S. cremoris population had fallen to less than five percent of the original number, while the S. lactis count remained at 42 percent of the original population. The five percent population level for the latter strain was not reached until the elapse of more than 12 weeks.

Gillies et al. (70) recently reported results from a survey of 109 vats of Australian Cheddar cheese in an attempt to determine the causes of a fermented flavor defect in the cheeses. These authors found no correlation between pH of the cheese or delayed acid production and the flavor defect. However, there was a relation between the appearance of the fermented defect and the early "die-out" of the starter organisms, with a simultaneous appearance of other Gram-positive microorganisms. In several instances, the

defect was associated with the use of two certain pairs of starters that contained S. lactis and S. cremoris strains. Bacteriophage and/or antibiotics were suggested as possible inhibitory agents which could have affected starter activity during late stages of cheese manufacturing and early ripening. Eleven percent of the examined cheeses developed the fermented defect; the off-flavor generally became apparent after about two months of curing. Vats of cheese produced with both mixed-strain commercial cultures and single-strain starter cultures exhibited this defect.

In 1966, Morris (136) summarized cheese quality gradings recorded for Queensland, Australia Cheddar cheese from 1962 to 1965. The persistence of a significant proportion of cheese below first grade (under 90 score) and the production of only an insignificant amount of choice grade cheese (93 score or higher) have been causes of continuing concern. Table 1 indicates the relative proportions of cheeses to which the most commonly used flavor-terms have been ascribed. It is interesting to note that the second most frequent flavor description was "fermented", with "unclean" flavor a very close third category for the total period. This is significant in light of a statement by Morris (136, 137) that "cheese graders sometimes interchange the terms fermented and unclean, thus causing the relative proportion of each to vary." For the above reason, neither of these two categories can be considered exclusive of the

Table 1. The percentages of Queensland, Australia Cheddar cheese graded into various flavor categories by experienced cheese graders.[a]

	Percentage of cheese according to flavor description					
	Flavor Criticism					
Year	Fermented	Unclean	Bitter	Weedy or feedy	Sour or acid	Other
1962/63	18.36	9.97	27.67	7.95	3.02	33.04
1963/64	13.87	13.98	19.22	7.19	5.16	40.56
1964/65	13.62	13.26	16.86	7.41	2.82	45.94
3 Year Average	15.28	12.40	21.25	7.52	3.67	39.85

a From Morris (136, p. 147).

Table 2. The percentages of Queensland, Australia Cheddar cheese graded below first grade according to type of off-flavor.[a,b]

	Percentage of cheese according to flavor description					
	Flavor Criticism					
Year	Fermented	Unclean	Bitter	Weedy or feedy	Sour or acid	Other
1962/63	43.6	25.7	3.6	14.9	9.5	3.0
1963/64	35.9	31.1	3.2	18.1	8.8	2.9
1964/65	50.3	28.3	1.6	13.4	5.1	1.3
3 Year Average	43.3	28.4	2.8	15.4	7.8	2.4

a From Morris (136,p. 148).

b Cheese below 90.0 score not considered as first grade.

other and they should be considered in association. As seen from Table 1, the total percentage of cheese graded into these two categories varied very little over the report period. Table 2 shows the distribution of cheese gradings below first grade, according to type of off-flavor. Nearly one-half of the low grade cheese in this Australian study was criticized for having a "fermented" flavor. This is an indication of the magnitude of the fruity and/or fermented defect to the cheese industry.

An intensive investigation of fruity flavor Cheddar cheese was recently conducted by Vedamuthu and co-workers (193, 194, 195). These workers selected certain starter cultures that showed tendencies to produce the fruity flavor defect consistently and used these in cheesemaking trials. The culture combinations employed by Vedamuthu, Sandine and Elliker (194) produced the following results in cheese:

Culture	Bacterial Species	General Cheese Flavor Result
A	16 S. cremoris strains and several Leuconostoc strains	Normal, or slightly acid, high quality
B	4 S. lactis, 2 S. cremoris and 1 Leuconostoc strain	Infrequently slight fruity/ fermented
C	3 S. lactis, 1 S. cremoris and 3 S. diacetilactis strains	Usually definite fruity/ fermented

Vedamuthu et al. (194) demonstrated that the starter organism population in the cheeses manufactured with cultures B and C persisted at relatively high levels for an extended period. These particular lactic streptococci apparently inhibited the normal development of the adventitious lactobacilli. In contrast, the use of culture A resulted in rapid decreases of the starter organisms in the cheese and the appearance of lactobacilli within eight weeks. The amount and type of carbonyl compounds produced by cultures A and C grown in milk were compared. This phase of the study revealed that culture C produced higher concentrations of formaldehyde, diacetyl and possibly pyruvic acid. These authors noted that the fruity flavor defect is usually accompanied by the slit-openness defect, probably due to the production of extensive amounts of gas during the ripening period. This entrapped gas apparently then produces open fractures between the curd particles, which appear as open slits.

Sherwood (174) produced a fermented flavor defect in Cheddar cheese by an additional inoculation of lactobacilli, isolated from mature cheese, into the cheese milk. Dodson, Hammond and Reinbold (53) encountered bitter and fermented flavors in starter-free cheese made with gluconic acid lactone. However, when selected strains of lactobacilli and ten ppm of manganese were added to the cheesemilk, the lactone-made cheeses possessed a good flavor and matured more rapidly than the control cheese. Several yeasts

isolated from cheese samples and subsequently grown in milk produced fruity odors (80). Nordström (143) has recently reported ester production by yeasts.

More recently, Bills (15) completed an investigation of the various components and certain starter organisms believed to be responsible for the fruity flavor defect of Cheddar cheese. In this study volatile constituents were isolated by distillation techniques from a typically fruity Cheddar cheese. The volatile constituents were subsequently separated by GLC and the components with frutiy odors determined by smelling the effluent stream of the column. The identity of components with fruity odors was subsequently established by mass spectral analysis. The only compounds isolated that possessed detectable fruity aromas were ethyl butyrate, ethyl hexanoate and ethyl octanoate. In this study Bills (15, p. 166) analyzed four cheeses possessing varied degrees of the defect and their matching non-fruity controls for quantitative amounts of certain key compounds. The results of his study on a semi-quantitative basis are summarized as follows:

Compound	Fruity Cheese	Non-fruity Cheese
Ethanol	400 to 2,040 ppm	36 to 320 ppm
Ethyl butyrate	1.6 to 24 ppm	0.7 to 4.7 ppm
Ethyl hexanoate	0.9 to 25 ppm	0.3 to 2.2 ppm

Bills et al. (19) suggested that the quantity of ethanol present in the

cheese may very well determine the amount of esters formed. This is evidenced by the correlation between high levels of ethanol, ethyl butyrate and ethyl hexanoate in the fruity cheese.

In his study, Bills (15, p. 90) demonstrated that the starters incriminated in the fruity defect produced considerably more ethanol than cultures which produced normal cheese when these cultures were incubated at 7° C. This is the temperature generally used for cheese curing. The above experimental observations support the hypothesis that certain cultures are directly responsible for the fruity flavor defect.

Unclean

An unclean flavor in Cheddar cheese is difficult to describe because it often varies in intensity and tends to lack a definite taste character (142, p. 211). However, this off-flavor lingers in the mouth of the taster long after the sample has been expectorated.

A number of investigators have suggested a possible correlation between certain nitrogenous compounds and unclean flavors in cheese. Silverman (176) reported that unclean flavors in Cheddar cheese appear to be related to high levels of cadaverine, putrescine and gamma-aminobutyric acid. Silverman and Kosikowski (180) found that cadaverine and putrescine were present in definite quantities in raw milk cheese, but these compounds were present at only

very low concentrations or not at all in pasteurized milk cheese.

Recently, Kosikowski and Fox (103) successfully reduced the number of colon-type bacteria which had been added to cheese milk by supercentrifugation (10,000 g. at 54.4° C). This treatment prevented the development of any unclean flavor in the cheese. Matching lots of Cheddar cheese were manufactured from milk inoculated with the same strains of E. coli and Aerobacter aerogenes and heated to 54.4° C prior to cheesemaking, and unclean-flavored cheese resulted.

Gillies et al. (70) reported that vats of Cheddar cheese which showed retarded acid development were degraded due to definite unclean and fermented off-flavors after only two months of ripening.

Microflora Associated with Cheddar Cheese Manufacture and Ripening

In discussing the microflora associated with Cheddar cheese, it is customary to group them as either "starter microorganisms" or adventitious microflora. Lactic streptococci are conventionally utilized as the starter culture in Cheddar cheese manufacture. The adventitious flora include all microorganisms that are unintentionally introduced into the cheese either through the milk supply, from equipment or from the environment. It is most difficult for the cheesemaker to control the adventitious flora, unless aseptic

conditions of manufacture are applied (122, 154). Therefore, the results of many manufacturing trials to test selected microorganisms as starter cultures are generally inconsistent or not well-defined, due to the difficulty encountered in controlling the varied, ubiquitous, adventitious flora. The consensus among cheese authorities is that the incidental microorganisms are essential to the cheese ripening process, but their exact role is difficult to determine.

The Role of Starter Bacteria

It is universally accepted that the chief function of the starter bacteria is to develop lactic acid at a satisfactory rate to carry out the cheese making process. Wilster (203, p. II-31) listed the reasons for using starter in Cheddar cheese manufacture. These are as follows:

1. To govern the flavor, body and texture of the cheese.
2. To aid rennet action through the acid produced.
3. The produced acid aids moisture expulsion.
4. The fermentations favorably affect changes in the curd during cheddaring and ripening.
5. The growth of undesirable bacteria in the curd and cheese is checked.

The conventional Cheddar cheese starter cultures generally consist of strains of S. cremoris, S. lactis and S. diacetilactis either singly or in combination. The United States cheese industry tends to prefer mixed-strain starter cultures supplied by commercial sources, whereas the majority of Australian and New Zealand cheese plants

and many Canadian and European factories employ single-strain starter cultures.

Walter et al. (199) suggested the use of a nonhemolytic strain of Streptococcus durans as a Cheddar cheese culture. The main advantage cited for this starter was the reduction of the manufacturing time by three to four hours. This process has not been utilized commercially, however, probably due to atypical body and texture development. By utilizing a higher cooking temperature of 106° F and Streptococcus thermophilus as the starter culture, Feagan (61) reported that good quality cheese was produced. Dahlberg and Kosikowski (38) experimented extensively with several strains of Streptococcus faecalis as a starter culture and as an additional microorganism to the conventional starter culture. The latter approach resulted in cheese with the highest score.

The comprehensive study by Vedamuthu (193) has already been mentioned. Findings reported by Vedamuthu et al. (194) indicated that the species and strains of microorganisms employed in the starter culture can either directly or indirectly affect the flavor of the cheese. They observed that S. cremoris cultures generally produced good quality cheese, whereas S. lactis and/or S. diacetilactis strain-containing cultures tended to produce fermented or fruity cheese.

In the previous discussion of the bitter flavor defect of cheese,

reference was made to the limited proteolytic ability of certain lactic streptococci, which permits the accumulation of polypeptides from cheese protein breakdown. In fact, Emmons et al. (55, 57) have classified various strains of S. cremoris as "bitter" or "non-bitter", according to their respective tendency to produce bitter-tasting peptides in Cheddar cheese. The activity of bacterial proteolytic enzymes has been studied by Czulak (30), Emmons et al. (56), Dawson and Feagan (44), Stadhouders (185) and Jago (93).

Kelly (101, p. 3) used strains of S. lactis and S. cremoris and a commercial starter separately as cultures for cheese manufacture in order to evaluate their effect on the resultant cheese. He observed that S. lactis cultures gave higher bacterial counts than S. cremoris starters, although there was little difference in cheese quality or the rates of protein-hydrolysis. Other cheese bacteriologists have studied the roles of S. lactis, S. cremoris, Leuconostoc citrovorum and Leuconostoc paracitrovorus in Cheddar cheese quality development, but no profound conclusions were established (5, 51, 76).

The Role of Adventitious Microorganisms

The Lactobacilli. Many cheese investigators have speculated on the role of lactobacilli and other adventitious microorganisms in the development of typical Cheddar cheese flavor. In 1914, Evans, Hastings and Hart (59) reported the presence of L. casei in Cheddar

cheese. In addition to this early work, Johns and Cole (95) observed that the numbers of lactobacilli were very low in fresh cheese, but that the bacterial counts for this organism generally increased after several weeks of curing. Other work has shown that the lactobacillus count of cheese generally reaches a maximum at three to six months of age (95), after which time the starter organism population usually declines (59). Franklin and Sharpe (66), Smith and Cunningham (182), Naylor and Sharpe (140, 141) and Perry and Sharpe (155) have subsequently confirmed the occurrence of L. casei in ripened Cheddar cheese.

Various workers have isolated and identified Lactobacillus plantarum (173), Lactobacillus brevis (66, 139, 140, 155, 182), Lactobacillus bulgaricus (84), Lactobacillus fermenti (155) and Lactobacillus helveticus (155). Some of the latter mentioned microorganisms tend to occur in ripening Cheddar cheese sporadically and probably are not typical microflora.

Mabbit (122), Vedamuthu (193), and Marth (126) have recently reviewed the possible role or associatiation of lactobacilli in the development of Cheddar cheese flavor. Mabbit (122) emphasized that the role of lactobacilli in Cheddar cheese is still obscure. He suggested that further carefully controlled inoculation experiments, with a study of the associative growth of streptococci and lactobacilli, would possibly be rewarding.

Feagan and Dawson (62) reported that 80 percent of the non-starter flora in Cheddar cheese consisted of lactobacilli. Allen and Knowles (5) found that most lactobacilli isolated from ripening Cheddar cheese were unable to grow vigorously in milk because a suitable nitrogen source was missing. They suggested that such compounds could be added to cheese milk. The slow growth rate of lactobacilli in Cheddar cheese has been partially explained by the restricted supply of carbohydrate, by the slow release of easily utilized energy sources and other inhibitory properties of the environment in cheese (111, 124).

Many studies have been conducted in which cheese milk was fortified with cultures of L. casei. Lane and Hammer (114), Sherwood (172), McDonald (129), and Yates, Irvine and Cunningham (207) achieved either flavor improvement and/or a higher Cheddar flavor intensity by the addition of L. casei strains to cheese milk or curd. Conversely, when Tittsler et al. (189, 190) and Yamamoto, Asao and Chikuma (205) studied the effect of L. casei added to cheese milk, the resultant cheeses were generally of lower quality than uninoculated controls.

Peterson and Johnson (157) demonstrated active lipolytic activity for L. casei cultures after 60 days of growth. These observations suggested that intracellular lipases were possibly involved, hence the authors postulated such enzymes may be involved in the

development of volatile fatty acids during later stages of Cheddar cheese ripening. Baribo and Foster (13) isolated proteolytic enzymes from disrupted cells of L. casei. Studies of these proteolytic enzymes were also conducted by Brandsaeter and Nelson (21, 22) and by Kristoffersen and Nelson (111, 112). The latter workers also reported that various strains of L. casei can produce hydrogen sulfide (111). Sharpe and Franklin (170) have also reported on the ability of L. casei to produce hydrogen sulfide.

Numerous investigators have reported the presence of L. plantarum in Cheddar cheese (66, 139, 140, 141, 155, 172, 182). Some strains of L. plantarum apparently tend to produce objectionable flavors and discoloration; whereas others either improved the flavor or have no effect. Sherwood (172) found that the addition of L. plantarum to cheese milk at a level that might be encountered in raw milk produced cheese with an improved flavor. Tittsler et al. (189, 190) and Ozawa and Kembo (146) conducted experimental trials by adding this organism to the lactic starter in cheese milk. Flavor developed early and substantial levels of volatile acids were produced. In a study of commercial Cheddar cheese by Sharpe and Franklin (170), 67 percent of the L. plantarum cultures isolated were found to be capable of producing hydrogen sulfide.

Naylor and Sharpe (139, 140), Perry and Sharpe (155), Smith and Cunningham (182) and Franklin and Sharpe (66) have confirmed

the appearance of L. brevis in Cheddar cheese. Most investigators have found that the inoculation of L. brevis into cheese milk resulted in Cheddar cheese with objectionable flavors, such as yeasty (35, 189).

Deane and Anderson (51), Deane et al. (52) and Tittsler et al. (189, 190) in separate investigations concluded that additions of L. bulgaricus to cheese milk made little, if any, contribution to cheese flavor. However, Yamamoto et al. (205) reported an improvement in flavor through addition of L. bulgaricus.

Bottazzi (20) and Annibaldi (7) have presented data which show that certain strains of L. helveticus possess proteolytic activity. Ozawa and Kembo (146) found that this lactobacillus grew during late stages of ripening and produced a sour cheese, but Tittsler et al. (189, 190) found that L. helveticus disappeared from cheese within two weeks and contributed little or nothing to proteolysis and flavor improvement.

Additions of Lactobacillus lactis to cheese milk in tests by Tittsler et al. (189, 190) resulted in no beneficial proteolysis or flavor development. Szaba and Balatoni (188) recently reported that a starter consisting of L. lactis and S. thermophilus was preferable to conventional lactic starters for Cheddar cheese manufacture. Bottazzi (20) utilized several highly proteolytic strains of L. lactis in cheesemaking, and claimed that results were

encouraging for application purposes.

Lactobacillus fermenti was one of the lactobacilli isolated by Perry and Sharpe (155) from raw milk and cheese subsequently made from the same milk. No cheese flavor development was associated with this microorganism. Fortification of cheese milk with L. fermenti by Tittsler et al. (189, 190) resulted in cheese with objectionable off-flavors and gassiness in late stages of ripening.

Lactobacillus acidolphilus was found to have little or no effect on proteolysis or cheese quality in the comprehensive tests by Tittsler et al. (189, 190). Sharpe and Franklin (170) found that this lactobacillus species is able to produce hydrogen sulfide under certain conditions.

The Micrococci. The presence of micrococci has frequently been reported in Cheddar cheese (3, 59, 62, 87, 89). As early as 1914, Evans et al. (59) observed the presence of micrococci in Cheddar cheese, and this has been recently verified by Irvine and Beach (89). Harris and Hammer (78) studied the effect of added micrococci to pasteurized cheese milk. They concluded that micrococci may assist normal Cheddar flavor development, but the organisms must be selected on the basis of proven strains and not by their species. Deane and Anderson (51), Robertson and Perry (163), Feagan and Dawson (62) and Peterson and Johnson (157) are other workers who have studied the role of micrococci in Cheddar cheese.

Alford and Frazier (3) observed that 78 percent of the non-lactic bacteria in good quality raw milk cheese consisted of strains of Micrococcus freudenreichii, Micrococcus caseolyticus and Micrococcus conglomeratus.

Various workers have demonstrated proteolytic potential (13) and lipolytic activity (157) for certain strains of micrococci. In light of the aforementioned findings and their rate of occurrence in Cheddar cheese, it is quite probable that their importance to cheese flavor development has lacked sufficient attention. Mabbit (122) stated that this possible oversight stems from the difficulty encountered in classifying and enumerating these microorganisms in the presence of the predominating lactic flora.

Other Microorganisms. The microflora of New Zealand Cheddar cheese was examined by Dacre (34, 37) in 1958. A significant number of the isolated microorganisms were identified as pediococci, which were approximately one-fourth as numerous as the lactobacilli throughout the ripening period. Franklin and Sharpe (66) and Perry and Sharpe (155) also encountered bacteria of the genus Pediococcus. In 1966, Fryer and Sharpe (67) reported the isolation of 59 strains of pediococci from a series of experimental Chedar cheeses. Unexpectedly, strains of Pediococcus cerevisiae were the predominant non-starter flora isolated from the cheese over a period of 18 weeks. All the pediococci strains required folinic acid for growth, which was

shown to be produced by the S. cremoris starter culture used in the experiments. The literature does not mention the isolation of pediococci species from Cheddar cheese made in the United States.

Clark and Reinbold (28) conducted a survey of ten Iowa cheese plants and studied the low-temperature microflora in 41 samples of young Cheddar cheese. More than one-half of the microorganisms isolated were enterococci. Of this group 60 percent were identified as S. durans, 27 percent as S. faecalis, 10 percent as S. faecalis var. liquefaciens and 3 percent as S. faecalis var. zymogenes. The authors noted a possible correlation between good Cheddar cheese (no flavor defects or body and texture abnormalities) and the level of enterococci recovered from the cheeses. Earlier reference has been made to the successful use of certain enterococci as cheese starters for flavor enhancement by Dahlberg and Kosikowski (38).

Occurrence of Slit-open Defect in Cheddar Cheese

Production of gas in the interior of the cheese during ripening frequently leads to a condition called slit-openness and/or sweet curd holes (126; 142, p. 206). Early production of gas in the cheese vat or in newly made cheese has been associated with coliform bacteria (29, 58). Coliform bacteria are frequently present in high numbers in raw milk and may readily be introduced to pasteurized

cheese milk from contaminated pipelines and vats.

In 1895, Russell (164) reported on the gas producing bacteria of cheese. He believed that the gas producers were lactic acid bacteria that liberated carbon dioxide and hydrogen during the fermentaion of lactose.

Bacillus coli communis-type ortanisms were noted by Moore and Ward (133, p. 125) to cause gas and taint production in Cheddar cheese. Marshall (125, p. 193-205) incriminated a similar organism of the colon group as being responsible for gassy cheese. Davis (41, p. 189) stated that A. aerogenes is the type of coliform that generally causes "gassy curd", whereas E. coli strains are chiefly responsible for certain off-flavors and taints.

The slit-openness defect has been associated with rapid carbon dioxide producing lactobacilli. In this study by Sherwood (172), the incriminated species for the defect were L. brevis, L. casei, L. plantarum and betacocci.

In studies by Albrecht and Ashe (1), the presence of certain heterofermentative starter streptococci were linked with slit-openness. They observed that some curd particles failed to fuse properly, and if such a condition was accompanied by the production of excessive amounts of carbon dioxide, the developed pressure was probably sufficient to cause curd particles to split apart at their contiguous surfaces. Another report involving heterofermentative

lactic acid bacteria with this defect was made by Overcast and Albrecht (145). They isolated L. citrovorum from open-defect Cheddar cheese. When this culture isolate was used in combination with a strain of S. lactis for cheese manufacture, typical "split-openness" occurred, but when the S. lactis strain was used alone, the cheese was free of the defect.

Vedamuthu (193, p. 135) measured the carbon dioxide producing ability of three S. diacetilactis strains isolated from a mixed-strain starter that consistently produced severe split-openness in experimental Cheddar cheese. He found that the three strains produced copious amounts (more than 700 microliters) of carbon dioxide in four hours at 30° C when grown in an 11 percent nonfat milk medium.

The Biochemistry of Cheese Cultures

Most cheesemakers and cheese microbiologists agree that the starter culture is the heart of cheesemaking. A defective starter is almost certain to produce a defective cheese, whereas a properly selected and prepared starter may actually overcome shortcomings such as poor quality milk or improper manufacturing techniques or curing practices.

The production of lactic acid from lactose is the primary function of starter bacteria, but complex metabolic processes are

involved. This results in the formation of many different compounds from various metabolites. It is most difficult to carefully study the biochemical activities of microorganisms involved in associative growth in such complex biological systems as milk or cheese. The species of bacteria encountered in this investigation utilize a number of different fermentation pathways for carbohydrate conversion. Hence, a discussion of the metabolic processes for homofermentative and heterofermentative classes of lactic acid bacteria is pertinent.

Homofermentative Lactic Bacteria Metabolism

The homofermentative bacteria produce lactic acid as the principal terminal catabolic product from glucose. Lactose is hydrolyzed to yield glucose and galactose. Glucose is readily metabolized to two molecules of pyruvate via the Emden-Meyerhof-Parnes (EMP) pathway (144, p. 240) and pyruvate is subsequently reduced to lactic acid. Galactose must first be converted to a glucose-1-phosphate by means of three enzyme catalyzed reactions in order to undergo catabolism (97).

Most homofermentative lactic streptococci can convert 70-90 percent of the fermented lactose to lactic acid. Kandler (97) has indicated that homofermentative bacteria apparently possess

enzymes which can oxidize and decarboxylate glucose-6-phosphate to ribulose-5-phosphate. Such biochemical activity could explain the formation of compounds other than lactic acid by this group of microorganisms.

Homofermentative bacteria related to Cheddar cheese manufacture and ripening are S. cremoris, S. lactis, S. diacetilactis, S. faecalis, S. durans, S. thermophilus, S. liquefaciens (160), L. casei, L. plantarum and L. helveticus (64, p. 17).

Platt and Foster (160) reported that a strain of S. cremoris produced acetic acid, formic acid, carbon dioxide and ethanol in addition to lactic acid. In the same study a strain of S. lactis formed acetic acid, carbon dioxide, ethanol and acetoin. These authors speculated that carbon dioxide evolves from the conversion of pyruvic acid to acetaldehyde. Gunsalus and Wood (75) found active alcohol dehydrogenase activity in certain homofermentative streptococci. In view of the observed conversion of aldehydes to their corresponding alcohols, Morgan and co-workers (134) concluded that S. lactis var. maltigenes apparently possesses a yeast-like alcohol dehydrogenase. The production of acetaldehyde by lactic streptococci has also been reported by Harvey (82), Lindsay, Day and Sandine (121), Keenan et al. (99) and Badings and Galesloot (9, Vol. 3, p. 199-208). Harvey (82) and Keenan et al. (99) also observed acetone in cultures of lactic streptococci. More recently

Bills and Day (16) studied the reduction of added acetaldehyde and propanal to ethanol and n-propanol by cultures of lactic streptococci. The levels of compounds were measured quantitatively by a gas-liquid chromatographic technique. These authors concluded that certain strains of S. lactis, S. cremoris and S. diacetilactis possess alcohol dehydrogenase activity.

There is also data in the literature to suggest that some homofermentative lactic streptococci can utilize glucose via the Entner-Doudoroff pathway (161). This provides another pathway for formation of ethanol and carbon dioxide.

Heterofermentative Lactic Bacteria Metabolism

Leuconostoc sp. are classified as heterofermentative microorganisms because they produce several catabolic products from glucose; i.e. acetaldehyde, carbon dioxide and ethanol in addition to lactic acid. This group of bacteria produces small amounts of D(-)-lactic acid, which differentiates them from the homofermentative lactic bacteria, which form L(+)-lactic acid (68, Vol. D, p. 144). The metabolic pathway for hexoses followed by heterofermentative bacteria partially involves the common hexose monophosphate shunt (97). Heterofermentative organisms apparently lack the enzyme aldolase, which catalyzes the conversion of

fructose-1, 6-diphosphate to dihydroxyacetone phosphate and 3-phosphoglyceraldehyde (161) in the EMP scheme.

Citric Acid Fermentation

Diacetyl is considered as one of the most important compounds contributing to the flavor of cultured dairy products. Several workers have reported that it could be significant in Cheddar cheese flavor, quite possibly as a constituent in the balanced component theory of Cheddar cheese flavor (24, 26). The formation of diacetyl from citric acid has been the subject of extensive, controversial research, and should be discussed.

Seitz (169, p. 91-96) has extensively studied the pathways for enzymatic conversion of citric acid to diacetyl and other compounds by S. diacetilactis strains. The mechanisms for citrate utilization by Leuconostoc sp. appear to be similar to the former microorganism, according to work reported by Galesloot (68, Vol. D, p. 153). Marth (126) has summarized the work and theories on the formation of diacetyl and acetoin. Pyruvic acid and alpha-acetolactic acid are the key intermediates and precursors, respectively, in the simultaneous fermentation of lactose and citrate to acetoin and diacetyl. One important aspect in this biochemical activity is that sufficient reduced nicotinamide adenine dinucleotide (NAD) is produced in the EMP scheme to reduce pyruvate to lactic acid. However, in the

fermentation of citrate, pyruvate is produced without a simultaneous supply of reduced NAD, therefore products besides lactic acid are formed. Most recently, Speckman and Collins (183, 184) substantiated that S. diacetilactis, strain 18-16 and a strain of L. citrovorum, synthesizes diacetyl from the acetaldehyde thiamine pyrophosphate (TPP) complex (from pyruvate) by attacking the carbonyl carbon of acetyl-CoA. They reported that neither acetoin nor alpha-acetolactate is the precursor of diacetyl for these organisms.

Ester Production by Microorganisms

A psychrophilic organism, Psuedomonas fragi, frequently is responsible for a fruity flavor defect in milk, cream and cottage cheese. Pereira and Morgan (152) observed a fruity aroma in milk cultures of P. fragi. They found that the fruity components were predominantly esters of isovaleric and acetic acids. They also concluded that hydroxamate-detected esters were probably ethyl esters. The production of ethanol by the organism or addition of ethanol to the culture prior to incubation appeared to govern the rate of ester formation.

As early as 1914, Hart et al. (81) studied the formation of esters in Cheddar cheese. Certain strains of streptococci and lactobacilli isolated from ripening cheese were able to produce esters and alcohols in milk cultures. They also noted that esters

did not appear in Cheddar cheese until the elapse of about five weeks. This observation compares very closely with recent observations of the first signs of the fruity defect in aging Cheddar cheese and the corresponding detection of elevated levels of esters (18). After conducting a series of experiments with dilute aqueous solutions of ethanol and acetic acid, Hart et al. (81) concluded that the esters probably were formed via biological activity only. They did not overlook the possibility that the acids or alcohols might be associated in some manner with the cheese constituents so that they could be brought together to facilitate ester formation.

EXPERIMENTAL

Cheese Manufacture

For this study approximately 54 vats of experimental Cheddar cheese were manufactured on a pilot-plant scale in the Oregon State University Dairy Products Laboratory. The cheeses were usually made in lots consisting of three vats per lot. Except for manual agitation of vat contents, commercial cheesemaking practices were closely followed. The method of cheddaring was by the Wilson technique (203, p. IV-6). Figure 1 is a flow diagram of the manufacturing steps and cheese grading procedure employed in this study.

Heat Treatment of Cheese Milk

High quality bulk milk produced by the Oregon Agricultural Experiment Station dairy herd was flash-heated at 64° C for 17 seconds for approximately 50 percent of the trials. Raw milk or milk pasteurized at 73° C for 17 seconds was utilized for the remaining trials.

Cheesemaking Equipment

Three adjacent 100 gallon-capacity, stainless steel vats were used in all cheesemaking trials. Each vat was equipped with its own

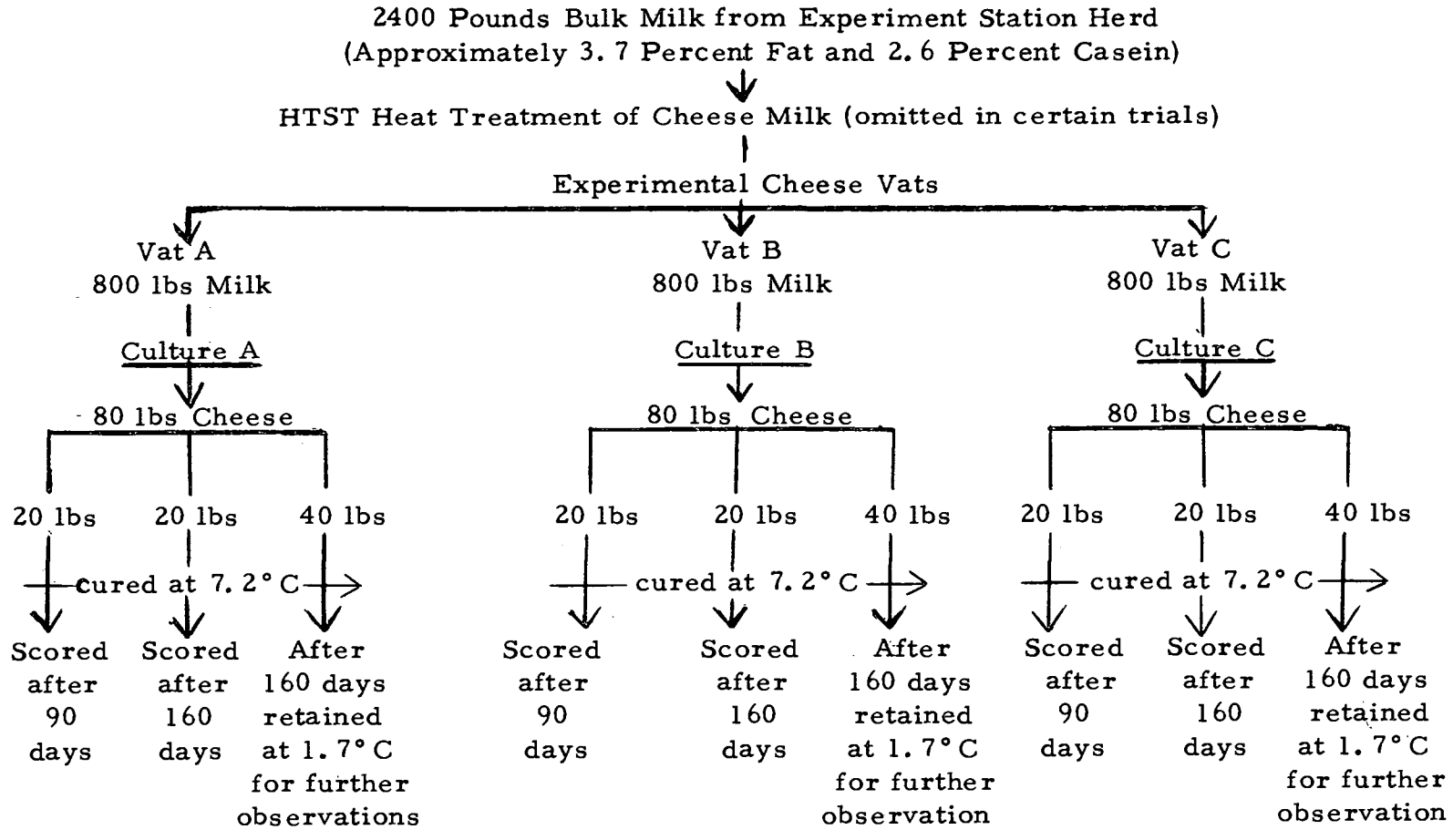

Figure 1. Flow diagram of the experimental procedure for the manufacture and scoring of cheese.

set of stirring paddles, cheese knife, strainer, fork, thermometer, and other equipment to prevent direct cross inoculation of microorganisms between vats. The vats, all equipment, and accessories and the hands of the cheesemaker were sanitized by immersion in a sodium hypochlorite solution (200 ppm available chlorine).

Cheese Cultures

Commercially lyophilized lactic streptococci were used to prepare bulk starters for cheese production. The cultures were selected partially on the basis of earlier studies by Vedamuthu (193, p. 43). The starters were prepared by direct inoculation of the lyophilized culture into sterile pretested reconstituted non-fat dry milk (11 percent solids). The starters were incubated at either 21° C or 30° C, depending upon the strain(s) in the respective cheese cultures. Following 18 hours incubation, the starters were titrated for acidity and either immediately added to cheese milk or held at 4.5° C until used. The coagulated starter was added to the vat in the amount of one percent by weight.

Manufacturing Conditions

The starter culture was added to the vat contents after the cheese milk had been heated to at least 21° C. Rennet was added at a temperature of 30° C one hour after the starter was added to

the cheese milk. A maximum cooking temperature of 40° C was used in the manufacturing trials. The cheddared curd slabs were milled when the whey attained approximately 0.55 percent acidity (expressed as percent lactic acid).

Salting, Hooping and Pressing of Cheese

The milled cheese curd was salted at a level of 2.5 percent by weight. The curd was agitated until the salt had thoroughly worked into the cheese. The green cheese yield was approximately ten percent of the original amount of milk. The curd was added either to one-40 pound cheese hoop and two-20 pound hoops, or to four-20 pound Wilson-style cheese hoops.

Cheese Curing

All the cheeses were cured at 7.2° C initially, then after 160 days of aging, the remaining cheese in each lot was held at 1.7° C until all evaluations and analyses of the cheese were completed. During the course of the investigation, portions of the partially aged cheese were moved and held at -23.4° C to prevent further enzymatic and microbial activity prior to the chemical analyses.

Grading of Experimental Cheeses

A panel of three or four expert cheese judges scored the

experimental cheeses after 90 days of curing and again after 160 days and/or one year of ripening. Portions of some experimental lots of cheese were held for up to two years at 1.7° C for further observation. The American Dairy Science Association (A.D.S.A.) cheese score card (192, p. 271) was used as a scoring guide by the panel to evaluate the cheese for body, texture and flavor.

Flavor Panel Evaluations of Cheese

Three laboratory flavor preference tests were conducted by the Sensory Evaluation Section of the Food Science and Technology Department. The four samples in each test included both experimental and commercial cheeses. The samples were served in coded cups to the judges seated in individual testing booths. From 170 to 180 University students served as judges and scored the samples on a nine-point hedonic scale from 1 (dislike extremely) to 9 (like extremely).

Taxonomic and Cultural Studies of Lactic Streptococci

Taxonomic Studies

Many of the taxonomic tests proposed by Sandine, Elliker and Hayes (165) and Mikolajcik (131) were performed to distinguish, separate and/or confirm the component strains of lactic streptococci

used in this investigation. Activity tests for all bacterial cultures were conducted by the Horral-Elliker method (86).

Maintenance of Cultures

All cultures were grown in pretested 11 percent solids non-fat milk medium (Matrix Mother Culture Media, Galloway-West Co., Fond Du Lac, Wisconsin). This medium was autoclaved for ten minutes at 121° C following reconstitution. Viability was maintained by transferring every third day, using a one to three percent inoculum. Most cultures were incubated at 21° C until coagulated (15 to 18 hours) or at 30° C for 15 hours. After incubation, all cultures were cooled and held at 2° C.

Acidity and pH Determinations

Titratable acidities on the cultures were determined according to the procedure conventionally used for dairy products, as outlined by Goss (74). All results were expressed as percent lactic acid. Either a Beckman Zeromatic or a Beckman Model G pH meter was used for all pH measurements. Samples were tempered to 21° C for measurement of pH.

Determination of Culture Proteolytic Activity

The proteolytic activity of various cultures was determined by

the method developed by Hull (88) for determining partial milk protein hydrolysis. The modifications suggested by Citti, Sandine and Elliker (27) were followed. Colorimetric measurements were made with a Bausch and Lomb Spectronic 20 spectrophotometer.

Microbiological Analyses of Cheeses

Cheese Sampling

Aseptic techniques were employed for obtaining representative samples of cheese curd and cheese from each experimental lot. Cheese trier plugs from a cross section of each cheese block were pooled to provide a representative sample.

Total Plate Counts

Five g of cheese were weighed into a sterile mortar and ground to a homogenous paste. A dilution blank containing 45 ml of two percent sodium citrate, tempered to 45° C, was gradually added to the mortar contents. The resulting uniform suspension was equivalent to a one-tenth dilution. Subsequent dilutions were made by transfer to sterile buffered distilled water blanks, prepared in accordance with Standard Methods for the Examination of Dairy Products (6, p. 67). The water blanks were also tempered to 45° C. Dilutions were plated on Elliker's lactic agar (54) and incubated at

30° C for 72 hours.

Microscopic Examinations

The cheese microflora was periodically observed by direct microscopic examination of methylene blue smears prepared from the one-tenth dilutions described above. For certain lots of cheese, 25 adjacent colonies were picked from a representative plate into sterile litmus milk and observed for coagulation, reduction, proteolysis, Gram-staining characteristics and morphology.

Methods for the Quantification of Acetaldehyde and Diacetyl

Determination of Diacetyl

The method of Pack et al. (147), with certain modifications, was used for all quantitative diacetyl determinations of cultures, cheese curd and ripened cheese. Aliquots of the culture or cheese being tested were weighed, rather than measured volumetrically. Pure diacetyl was used in place of dimethyl glyoxime for preparing the dilutions for the standard curve. All samples were read against a reagent blank at 530 mμ using a Beckman DU spectrophotometer.

Cheese or curd for diacetyl determinations was prepared by making an homogenous slurry. The cheese or curd sample was chilled to 0-2° C and then grated into a covered stainless steel pan.

Ten ml of 2 percent aqueous sodium citrate were added to a 25 × 250 mm culture tube and then 20 g of grated cheese were weighed into the tube. Ten ml of water and a small amount of anti-foam (spray-form) were added prior to unit assembly. The percent recovery of diacetyl from Cheddar cheese curd or ripened cheese was found to be 90 percent and all quantification data were corrected on this basis.

Determination of Acetaldehyde

Acetaldehyde in cultures and cheese was quantified by the 3-methyl-2-benzothiazalone hydrazone hydrochloride method described by Lindsay and Day (120). All samples were read against a reagent blank at 666 mμ using a Beckman DU spectrophotometer. This method determines total volatile aldehydes, but the work of Lindsay (119, p. 83) indicated that acetaldehyde comprised the major portion of the aldehydes in lactic cultures.

It was necessary to modify the method of sample preparation for cheese curd or ripened cheese to obtain a satisfactory recovery rate for added acetaldehyde. Sufficient concentration of cheese, limited loss of volatile material and adequate purging of sample with nitrogen were attained by making an homogenous slurry. The chilled cheese was grated into a covered stainless steel pan. Five ml of 5 percent aqueous phosphoric acid were added to a 25 × 250

mm culture tube and 15 g of grated cheese weighed into the tube. Ten ml of water and a small amount of antifoam agent (spray-form) were added before unit assembly. The percent recovery of acetaldehyde from acidified cheese slurries (pH 3.5) was found to be 65 percent. Quantification data were corrected on this basis.

Determination of Volatile Esters

The modified hydroxamic acid method adapted by Lindsay (119, p. 87) was used for the determination of volatile ester content of cheese curd. Cheese slurries were prepared in the same manner as for diacetyl determination. Absorbances were read at 525 mμ against a reagent blank using a Beckman Model DU spectrophotometer and were referred to a standard curve. The percent recovery of added ethyl acetate from prepared cheese slurries was found to be 80 percent and all data were corrected on this basis.

RESULTS AND DISCUSSION

Taxonomic and Cultural Studies of the Lactic Streptococci Used in this Investigation

During the course of this investigation it was necessary to confirm the species identify of all strains of lactic streptococci studied. Various cultural studies were conducted in an attempt to obtain data which could be useful in predicting the tendency for certain species or strains of lactic streptococci to develop the fruity or fermented flavor defect in Cheddar cheese.

Taxonomic Studies of Cheese Cultures

The various taxonomic tests proposed by Sandine et al. (165) were performed to separate and/or identify the component strains in the commercial starter cultures and confirm the identity of single-strain cultures. Results of the taxonomic studies and physiological characteristics of the component strains in the single-strain and mixed-strain cultures studied are listed in Table 3. Culture numbers 1 through 10 refer to the selected single-strain cultures and cultures A, B, and C are the mixed-strain cultures which were utilized for most cheesemaking trials.

Table 3. Cultural and physiological characteristics of the single- and mixed-strain cultures of lactic streptococci used in this investigation.

No.	Strain Code	Time (hours) to Coagulate Milk @ 21 °C	Time (hours) to Coagulate Milk @ 30 °C	Arginine Hydrolysis[a]	King's Test for Diacetyl	Growth in salt medium 4.0%	Growth in salt medium 6.5%	Species Identity
1	C-224a	18	6	+	+	+	-	S. diacetilactis
2	C-289a	18	6	+	+	+	-	S. diacetilactis
3	C-292	24	18	+	+	+	-	S. diacetilactis
4	18-16	-	21	+	+	+	-	S. diacetilactis
5	DRC,	24	12	-	+	+	-	S. diacetilactis
6	$M\text{-}21_{35}$	24	12	+	+	+	-	S. diacetilactis
7	11 D,	18	12	-	-	-	-	S. cremoris
8	$C\text{-}2_f$	15	12	+	-	+	-	S. lactis
9	8304	-	18	+	-	+	+	S. faecalis
10	ML-3P	15	12	+	-	+	-	S. lactis
Culture A		18		-	-		-	S. cremoris
Culture B		16		+	-		-	S. lactis, S. cremoris,[b] Leuconostoc sp.
Culture C		16		+	+		-	S. diacetilactis, S. lactis,[b] S. cremoris

a In Mikolajciks broth (131).

b Isolates from culture B and C subjected to the above taxonomic procedures to confirm the identity of component strains.

+ = Positive, - = Negative

Proteolytic Activity of Cultures

The Hull method (88) as modified by Citti et al. (27) was used to determine the extent of hydrolysis of milk protein by cultures utilized in the course of this investigation. The proteolytic activity of these cultures is shown by data in Table 4. The extent of proteolytic activity for the cultures was closely correlated with the level of acid development (pH), except for culture number 3, which developed moderate acidity, but demonstrated very little protein hydrolysis in milk culture.

Table 4. Hydrolysis of milk protein by lactic starter cultures as determined by the Hull method (88).[a]

No.	Organism	Coagulation	pH	μg Tyrosine/ml[b]
1	S. diacetilactis C-224	+	4.5	27.7
2	S. diacetilactis C-289	+	4.5	28.6
3	S. diacetilactis C-292	+	4.7	6.2
4	S. diacetilactis 18-16	-	6.2	2.0
5	S. diacetilactis DRC	+	4.6	32.3
6	S. diacetilactis M 21-38	-	5.8	3.5
7	S. cremoris 11 D	-	6.1	2.0
8	S. lactis C-2	+	4.5	29.2
9	S. faecalis 8304	-	5.8	2.7[c]

a Milk prepared by pasteurizing at 62° C for 30 minutes, inoculated at the rate of three percent and incubated for 18 hours at 21° C.
b Average of four trials.
c Incubated at 30° C for 18 hours.
+ = Positive, - = Negative

Study of Culture Activity

Vedamuthu (193, p. 73) established that certain mixed-strain starter cultures yield fruity or fermented cheese and attempted to identify the specific species and strains in the starters that were responsible for this defect. Several single-strains were found to be unsuitable for cheese manufacturing trials due to their inability to produce sufficient quantities of lactic acid in the cheese vat. In light of this limitation, the single-strain cultures utilized in this investigation were subjected to the culture activity test described by Horral and Elliker (86). The activity test results are found in Table 5. Only the cultures found to have a "fast" or "moderate" rate of acid development were considered to be satisfactory for cheesemaking trials. One strain of S. diacetilactis (number 4), a strain of S. cremoris (number 7) and the S. faecalis culture (number 9) were rated as unsuitable for cheese manufacture.

Acetaldehyde and Diacetyl Production

Nine of the single-strain cultures used in this study were analyzed for acetaldehyde production. The amounts of acetaldehyde produced by the cultures under standardized growth conditions are recorded in Table 6. Five of six different single-strain cultures of S. diacetilactis produced an average of 2.41 to 8.58 ppm of

Table 5. Simulated cheese vat activity tests for single-strain lactic cultures by the Horral-Elliker method (86).

No.	Organism	Titratable Acidity[a]			Activity[b] Rating
		A	B	Average	
1	S. diacetilactis C-1-224a	0.45	0.53	0.480	Fast
2	S. diacetilactis C-2-289a	0.55	0.60	0.575	Fast
3	S. diacetilactis C-3-292	0.40	0.41	0.405	Moderate
4	S. diacetilactis 18-16	0.24	0.25	0.245	Slow
5	S. diacetilactis DRC-1	0.40	0.44	0.420	Moderate
6	S. diacetilactis M21-35	0.41	0.46	0.435	Moderate
7	S. cremoris 11C-1	0.28	0.28	0.280	Slow
8	S. lactis C2-F	0.39	0.38	0.385	Moderate
9	S. faecalis 8304	0.19	0.18	0.185	Slow
10	S. lactis ML-3P	0.40	0.41	0.405	Moderate
11	Mixed Strains of S. cremoris	0.48	0.52	0.500	Fast

a Expressed as percent lactic acid.

b A culture that produced less than 0.35 percent acid = slow, 0.35-0.40 percent acid = moderate, and more than 0.45 percent acid = fast.

Table 6. Acetaldehyde production by single-strain lactic cultures.[a]

No.	Organism	pH	Acetaldehyde (ppm) A	B	C	Average
1	S. diacetilactis C-1-224a	4.58	5.37	4.63	5.63	5.21
2	S. diacetilactis C-2-289a	4.57	6.25	5.75	6.25	6.08
3	S. diacetilactis C-3-292	4.79	--	1.66	3.16	2.41
4	S. diacetilactis 18-16	6.12	4.12	3.62	--	3.87
5	S. diacetilactis DRC-1	4.65	7.24	9.25	8.26	8.58
6	S. diacetilactis M21-35	4.85	0.19	0.32	1.50	0.67
7	S. cremoris 11D-1	5.63	3.44	7.00	--	3.72
8	S. lactis C2-F	4.58	7.41	6.06	3.38	5.62
9	S. faecalis[b] 8304	5.30	4.62	--	--	4.62

a Incubated at 21° C for 18 hours.

b Incubated at 30° C for 18 hours.

acetaldehyde. S. diacetilactis strain M21-35 (number 6) produced only 0.67 ppm of acetaldehyde (average of three trials).

The ability of lactic streptococci to produce measurable quantities of acetaldehyde has been reported by Harvey (82), Lindsay et al. (121), Keenan et al. (99) and Bills and Day (16). The fact that the latter two groups of workers reported different concentrations of acetaldehyde than this author for several identical strains of lactic streptococci may be partially accounted for by varied growth conditions (i.e. incubation temperature and percent inoculum). However, the relative levels of acetaldehyde produced by the various species of lactic streptococci studied coincide with the concentrations of this compound reported by other investigators (16, 82, 99, 121).

The quantities of diacetyl produced by the single-strain cultures examined are listed in Table 7. The diacetyl concentrations, determined after 18 hours incubation at 21° C, ranged from 0.39 to 1.75 ppm for six strains of S. diacetilactis.

Manufacture of Experimental Cheeses

In the initial stages of work conducted by Vedamuthu (193), different commercial starter cultures were used for making Cheddar cheese. A definite relationship was found between the development of distinctive body and flavor characteristics in the cheese and the

Table 7. Diacetyl production by single-strain lactic cultures.[a]

No.	Organism	Diacetyl (ppm) A	B	C	D	Average
1	S. diacetilactis C-1-224a	0.66	1.25	0.89	0.86	0.92
2	S. diacetilactis C-2-289a	0.77	0.91	0.78	1.11	0.89
3	S. diacetilactis C-3-292	0.50	0.62	0.72	0.78	0.66
4	S. diacetilactis 18-16	1.34	2.19	1.72	--	1.75
5	S. diacetilactis DRC-1	1.39	1.52	1.10	--	1.34
6	S. diacetilactis M21-35	0.23	0.22	0.72	--	0.39
7	S. cremoris 11 D-1	0.04	0.09	0.05	0.05	0.06
8	S. lactis C2-F	1.10	1.25	--	--	1.18
9	S. faecalis[b] 8304	1.82	1.28	--	--	1.55

a Incubated at 21° C for 18 hours.

b Incubated at 30° C for 18 hours.

use of certain cultures. Specific patterns of body and flavor scores for the experimental cheese were noted by four judges upon evaluation of the cheese after curing for 90 or 160 days.

In this work, cheesemaking conditions were varied to study the possible effect on resultant cheese. In addition to different commercial starters or single-strain starters, various heat treatments of the cheese milk and chemical treatment of the milk were studied. Table 8 lists the various treatments of cheese milk, the culture combinations used in this investigation and the resultant flavor score and description for each lot of experimental cheese.

In the present investigation it was postulated that either certain strains of S. diacetilactis or strains of this organism growing in association with other lactic streptococci were a primary causative agent for the development of the fruity/fermented flavor defect in experimental Cheddar cheese. This basic hypothesis stemmed from observations of data presented by Vedamuthu (193, p. 61). A number of cheesemaking trials were conducted in an attempt to validate the above hypothesis (series 15 and 16, Table 8).

Effect of Cheese Milk Treatment

It was pertinent to this investigation to study the possible effect of raw milk, flash heated milk (64° C for 17 seconds) and pasteurized milk on the flavor development of experimental cheese, concomitant

Table 8. Type of treatment for cheese milk, culture combination and the resultant flavor score and criticism judgements for experimental Cheddar cheeses.

Series	Lot Code	Cheese Milk Treatment	Bacteria Culture Utilized[b]	Resultant Ave. Score[c]	Cheese Flavor Criticism
1	X-1A, X-2A	Flash heated control	Culture A	38.75	Slight acid and/or bitter
2	X-1B, X-2B	Raw, seeded	Culture A + S. lactis ML-3P	37.75	Slight fermented
3	X-1C, X-2C	Raw, Control	Culture A	38.25	Slight acid or bitter
4	X-3A, X-4A	Flash heated	Culture A	38.75	Acid or slight bitter
5	X-3B, X-4B	Flash heated	Culture B	37.00	Fermented, unclean
6	X-3C, X-4C	Flash heated	Culture C	36.75	Fruity, fermented and diacetyl-like
7	X-5A, X-6A	Pasteurized	Culture A	38.50	Acid, slight bitter and flat
8	X-5B, X-6B	Pasteurized	Culture B	37.00	Diacetyl-like, acid, fermented, unclean
9	X-5C, X-6C	Pasteurized	Culture C	37.50	Fermented, fruity diacetyl-like

Table 8. (Continued)

Series	Lot Code	Cheese Milk Treatment	Bacteria Culture Utilized [b]	Resultant Ave. Score [c]	Cheese Flavor Criticism
10	X-8 D-1	Flash Heated	Culture D	38.50	Acid
11	X-8 D-2	Raw, Seeded	Culture D + S. lactis ML-3P	38.00	Slight acid and fermented
12	X-8 D-3	Raw, Control	Culture D	38.50	Acid
13	X-9 C-1	Flash Heated	Culture C	37.50	Fruity/fermented
14	X-9 C-2	Raw, Seeded	Culture C + S. lactis ML-3P	37.25	Definite fruity
15	X-9 C-3	Raw Control	Culture C	37.50	Fruity/fermented and acid
16	X-10 E-1	Flash Heated	Culture E	39.25	Full Cheddar flavor
17	X-10 E-2	Raw, Seeded	Culture E + S. lactis ML-3P	35.25	Definite bitter and slight fermented
18	X-10 E-3	Raw, Control	Culture E	38.75	Slight bitter and whey taint

Table 8. (Continued)

Series	Lot Code	Cheese Milk Treatment	Bacteria Culture Utilized[b]	Resultant Ave. Score[c]	Cheese Flavor Criticism
19	X-11A, X-12A	120° F + H_2O_2 and Catalase	Culture A	39.25	Good Cheddar flavor
20	X-11B, X-12B	120° F + H_2O_2 and Catalase	Culture B or D	37.50	Acid, slight bitter and slight musty
21	X-11C, X-12B	120° F + H_2O_2 and Catalase	Culture C	36.50	Definite fruity/fermented
22	X-13A, X-14A	Pasteurized	Culture A	37.50	Acid and bitter, slight musty
23	X-14B	Pasteurized	Culture F	36.50	Slight acid and fermented
24	X-13C, X-14C	Pasteurized	Culture C or component S. lactis and S. cremoris of C	36.00	Fruity/fermented and unclean

Table 8. (Continued)

Series	Lot Code	Cheese Milk Treatment	Bacteria Culture Utilized [b]	Resultant Ave. Score [c]	Cheese Flavor Criticism
25	X-15A, X-16A	Pasteurized	Modified Culture A	39.00	Flat, slight acid
26	X-15B, X-16B	Pasteurized	M21-35 (No. 6)	38.50	Acid
27	X-15C, X-16C	Pasteurized	C-1-224a (No. 1)	38.75	Acid

a Flash heated at 64° C for 17 seconds; pasteurized at 73° C for 17 seconds.

b See Table 3 for component species and strains.

c Cheese flavor score expressed as average of replicate vats after 90 and 160 day gradings by panel of four experienced cheese judges. No criticism: 40 score, based on A. D. S. A. score card (192, p. 271).

with varying the starter cultures. Examination of series 5, 6, 8, and 9 (Table 8) indicates that the specific culture employed affected the flavor score and type of flavor independent of the extent of heat treatment.

Experiments (series 1, 2, and 3, Table 8) were performed to determine the effect of raw milk and raw milk seeded with S. lactis ML-3P on the flavor of the resultant cheese. The flavor of these cheeses was compared to those made from flash heated milk. The control culture (culture A) was employed as the cheese starter in all three vats for each of the two replicate lots in this experiment. The raw milk control and flash heated control vats of cheese (series 3 and 1) were assigned flavor scores of 38.25 and 38.75 respectively and each was criticized for having a slight acid and/or bitter flavor. The vat of cheese milk which had been seeded with S. lactis ML-3P (one hour incubation at 25° C) was criticized for having a slight fermented flavor and given a lower score of 37.75.

Series 19, 20, and 21 were performed to determine the effect of hydrogen peroxide-catalase treatment of cheese milk as described by Morris (135) on the flavor of the resultant cheese. Culture C tended to develop a definite fruity or fermented flavor as readily in this instance as in other series of experiments with this defective culture. The body and texture of the cheeses in this series tended to be slightly weak and pasty-bodied. However, the fruity cheeses

produced with culture C did not possess the characteristic slit-openness or sweet-curd hole defect so frequently associated with fruity and/or fermented Cheddar cheese. The above results indicate that fruity or fermented flavor can develop in Cheddar cheese made from chemically treated milk as well as from raw or heat-treated milk.

Effect of Specific Cheese Culture

S. lactis Cultures. It is most interesting to note that each experimental cheese which developed a fruity or fermented flavor defect contained at least one strain of *S. lactis*. In series 2, 11, 14, and 17 (Table 8) the control culture of *S. cremoris* strains plus a seed culture of *S. lactis* ML-3P was incorporated in the cheese manufacturing process. Each vat of cheese containing the latter organism developed a fruity and/or fermented flavor (average flavor score: 37.06). The average flavor score of all cheeses made with control cultures of *S. cremoris* was 38.84. The results obtained in this investigation from the use of *S. lactis* ML 3-P as a component of the cheese culture are comparable to the findings of Vedamuthu (193, p. 70) and Perry (153). Perry described the flavor as "dirty" or "fruity"; subsequently the term "lactis" flavor was derived to describe the off-flavor produced by three different strains of *S. lactis*. However, in the work of the latter author

cheeses made with strain ML-3P always exhibited a stronger fruity flavor than cheeses of corresponding ages made with S. lactis strains ML-2 or ML-8.

In the course of following the flavor development of experimental cheeses, it was noted that the intensity of fruity or fermented off-flavors increased as ripening proceeded. After about nine months aging, judges generally considered the flavor as fermented-unclean. Perry (153) recorded exactly the same observations, except that the term "dirty" flavor was used to describe the well-matured, defective cheese.

Cheese trial series 5, 6, 8, 9, 14, 15, 21 and 24 all developed the fruity/fermented flavor defect. Analysis of the component strains in these cheese cultures (Table 3) show that each culture employed in these vats contained at least one or more strains of S. lactis. This adds to the evidence for the incrimination of S. lactis as the species apparently responsible for the development of the fruity or fermented off-flavor in Cheddar cheese. The term "lactis" flavor, coined by New Zealand investigators, is probably therefore most appropriate.

S. diacetilactis Cultures. Examination of the flavor criticism for series 26 and 27 (S. diacetilactis used as the starter) shows that no fruity or fermented flavor developed in the cheeses within a 12 month observation period. Ten other vats of cheese were

manufactured in a commercial cheese vat with five different single- and mixed-strain cultures of S. diacetilactis during July and August, 1965. None of these vats of cheese developed a fruity or fermented flavor defect, although the fresh curd possessed a definite green or "yoghurt-like" flavor (9, Vol. B, p. 199) and the ripened cheese exhibited moderate levels of slit-openness.

The literature includes several reports on the use of S. diacetilactis strains in the manufacture of cheese. Swartling and Lindgren (187) studied the influence of S. diacetilactis and other aroma bacteria on the quality of Herrgård cheese. They found that these bacteria are responsible for the formation of the gas necessary for the normal texture and appearance of this type of cheese. Czulak (31) has reported on the use of S. diacetilactis in New Zealand Cheddar cheese manufacture. Vedamuthu (193, p. 98-99) has also studied the use of this species in cheese manufacturing. He found that this organism has the ability to withstand higher salt concentrations, that it persists longer than S. lactis and S. cremoris strains in ripening Cheddar cheese and that it apparently inhibits the growth of adventitious bacteria such as Lactobacillus species.

Cheesemaking and Grading Records

It should be emphasized that the experimental cheeses were manufactured under standard conditions and commercial cheese

manufacturing practices were adhered to as closely as possible with the pilot-scale vats and accessories available. Chart 1 in the appendix illustrates a "cheese make-sheet" for a typical lot (Lot X-6) of cheese manufactured during the course of the study. The chart contains information relating to the composition and bacterial content of cheese milk, a record of temperatures, titratable acidities and time elements involved in the manufacture of experimental cheeses. All cheeses were within the normal range of chemical composition and pH. It is necessary to obtain and record such manufacturing and analytical data, since it is difficult, if not impossible, to assess the significance of bacteriological findings unless such information is available.

Chart 1a (appendix) presents analytical data pertaining to gross composition of several typical cheeses. Chart 1b is a record of the flavor, body and texture scores and criticisms for the above vat of cheese after 90 days of ripening at 7.2° C. Similar records were maintained on all other lots of cheese.

Microbiological and Chemical Analyses of Experimental Cheeses

Plate Counts on Cheese

In earlier work on the relation of lactic streptococci to the fruity flavor defect of Cheddar cheese, Vedamuthu (193, p. 75-80)

noted certain trends in the microbial population in cheeses made with cultures A, B, and C. Cheeses made with cultures B and C demonstrated very gradual declines in total bacterial count compared to cheeses made with culture A. In addition, this worker noted that the total bacterial numbers were much higher in fruity defect cheeses than in normal control cheeses throughout the 90 day testing period. Similar assessments of bacterial counts for experimental cheeses were observed in this investigation. Table 9 shows the bacterial counts obtained at various stages of manufacture and ripening for Lot X-15. Figure 2 shows the bacterial population survival patterns in the various cheeses from initial manufacturing stages to eight weeks of age. The control culture of S. cremoris strains did not reach the high cell count achieved by the two S. diacetilactis experimental cultures. Observation of Figure 2 also indicates a more rapid disappearance of bacteria in the control culture cheese than in the cheese made with S. diacetilactis cultures. This is in agreement with the results of Vedamuthu (193, p. 76-80). Presumably, the rapid decline of bacterial cells in the cheese manufactured with S. cremoris culture can be attributed to the "die-out" of this species (62), due to limited salt tolerance and other cheese environment conditions. The above observations are especially interesting in view of the fact that no lactobacilli or micrococci were observed in cheese smears and none were isolated from plates

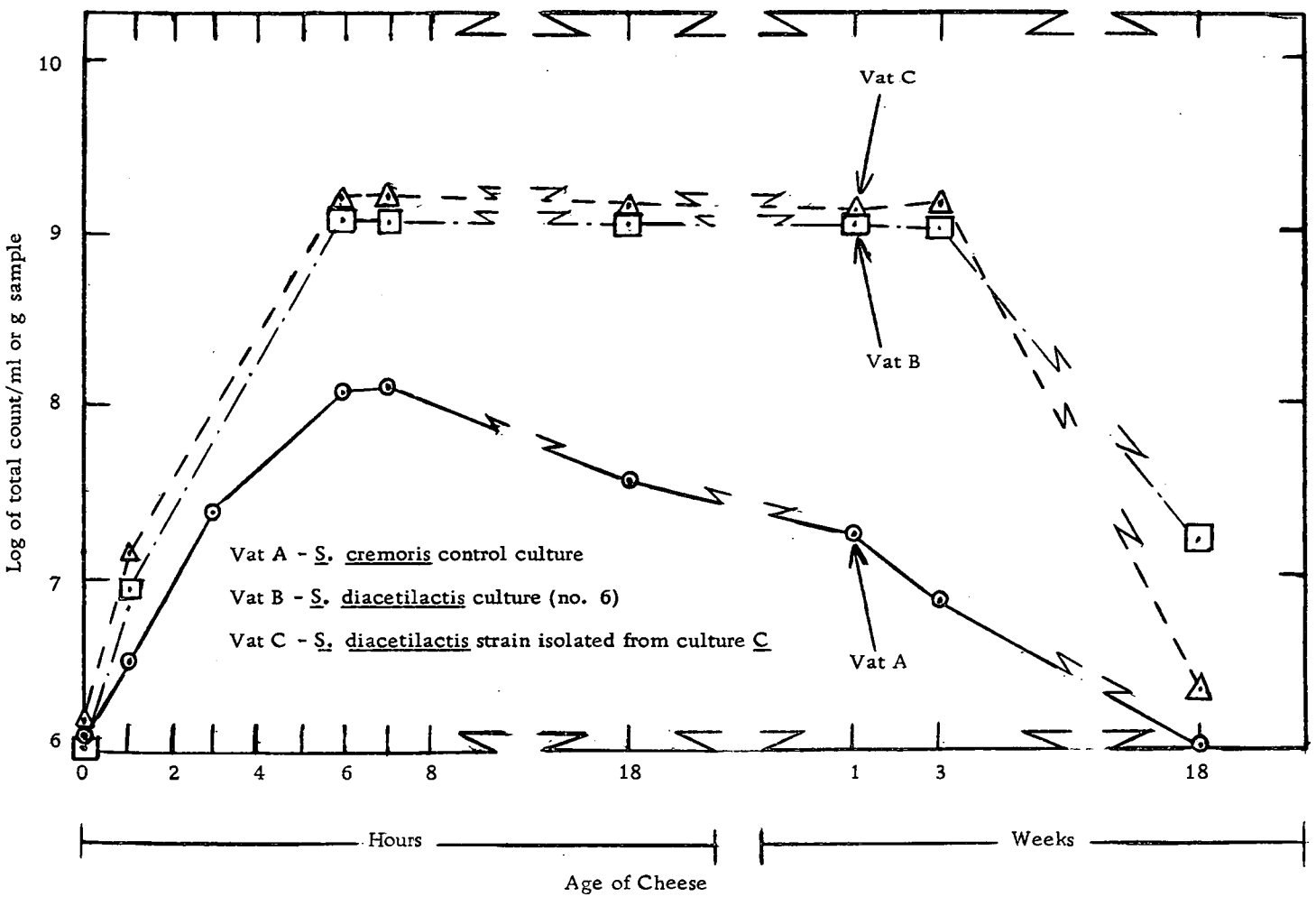

Figure 2. Bacterial growth and survival trends in cheeses from Lot X-15.

by picking and subculturing colonies during the course of an eight week ripening period. This implies that nearly all countable bacteria were primarily starter streptococci. Very similar results were obtained for Lot X-16, which was a replicate cheesemaking trial.

Table 9. Total bacteria counts for cheeses in Lot X-15, Vats A, B, and C at various stages of manufacture and ripening.[a]

Sample no.	Source of Sample	Bacteria Plate Count ($\times 10^6$)		
		Vat A	Vat B	Vat C
1	Starter inoculation[b]	12.5	11.9	17.3
2	After 1 hour incubation[b]	47.8	96.0	131.0
3	End of cooking period[c]	333.0	> 500	> 500
4	Curd packing	> 500	> 500	> 500
5	Mid-Cheddaring	> 500	> 500	> 500
6	Milling	1,210	4,300	> 5,000
7	Pre-pressing	1,430	3,960	> 5,000
8	Out-of-press	508	3,920	5,000
9	After one week aging	233	3,390	3,970
10	After 3 weeks aging	85	1,845	4,050
11	After 8 weeks aging	< 1	191	29

a Plated in duplicate on Elliker's lactic agar (54), incubated at 30° C for 72 hours.

b Milk sample.

c Sample numbers 3-11 were cheese curd.

Quantification of Acetaldehyde in Cheese

In view of the basic hypothesis that certain strains of S. diacetilactis were the causative agent for the fruity flavor defect, it was deemed appropriate to study the formation and subsequent accumulation or disappearance of acetaldehyde in cheese curd and ripening cheese. The ability of S. diacetilactis strains to produce measurable quantities of acetaldehyde has been well documented (16, 82, 99, 122, 9, p. 191-208).

The procedure for rapid quantification of acetaldehyde in lactic cultures described by Lindsay and Day (120) was modified and adapted for analysis of Cheddar cheese and fresh curd. Initially, extreme difficulty was experienced in attaining satisfactory recovery rates. It was found that the method of sample preparation, adjustment of pH and the nitrogen purging rate were critical factors in obtaining satisfactory results for acetaldehyde determinations on cheese curd or cured cheese. Table 10 shows the effect of the diluent and the pH of the cheese slurry on the recovery rate for known amounts of acetaldehyde added to cheese preparations. Mohammed, Olcott and Fraenkel-Conrat (132) studied the reactions of proteins with acetaldehyde and concluded that acetaldehyde readily combines with available amino and guanidyl groups, particularly in the pH range 7-8. The previously mentioned activity is not noted if the amino

groups are previously blocked. Decreasing the pH of cheese slurries may partially prevent binding of the acetaldehyde by the cheese protein, leaving the compound free to react with the test reagents. The use of five percent phosphoric acid as a diluent decreased the pH of cheese slurries to pH 3.5 and acetaldehyde recovery rates of 65 percent were then achieved.

Table 10. Effect of the diluent and pH on the recovery rate for added acetaldehyde to cheese slurry preparations.

Cheese Slurry Preparation[a]	pH	Percent Recovery
20 g cheese 9 ml 2% sodium citrate	5.39	2.4
20 g cheese 8.5 ml 2% sodium citrate 0.5 ml 2N acetic acid	5.30	4.7
20 g cheese 39 ml 2% sodium citrate	5.25	8.1
20 g cheese 39 ml distilled water	5.10	36.1
20 g cheese 10 ml 5% H_3PO_4 9 ml 2% sodium citrate	3.50	65.0
20 g cheese 19 ml 5% H_3PO_4	2.30	68.5

a Each preparation included one ml of a 5.0 ppm acetaldehyde solution.

The amounts of acetaldehyde recovered from Lot X-15 experimental cheeses are presented as part of the data in Table 11. Relatively high levels of acetaldehyde were detected in the S. cremoris (Vat A) and the S. diacetilactis (Vat C) bulk starters. Concentrations of acetaldehyde in these cultures were 6.25 ppm and 7.50 ppm, respectively. The level of acetaldehyde tended to gradually decline in each cheese at a fairly uniform rate with increasing age of cheese. The concentrations of acetaldehyde found in the cheese curd and the young cheeses is somewhat higher than the values recorded by Bills (15, p. 74) for ten samples of commercial Cheddar cheese. However, the cheeses examined by the latter author were more completely ripened and the method of determination was semi-quantitative. Bills (15, p. 77) emphasized that relatively low concentrations of acetaldehyde were isolated from the cheese samples in his study.

Six different fruity and fermented cheeses (160 days of age) were analyzed for acetaldehyde, but all samples contained less than 0.15 ppm of the compound. This is undoubtedly indicative that acetaldehyde concentration is not directly responsible for fruity/fermented flavor, nor an effective measure of the off-flavor. This observation is noteworthy in view of the original hypothesis that high acetaldehyde-producing strains of S. diacetilactis are directly or indirectly responsible for this defect in Cheddar cheeses. Conceivably, strains of lactic streptococci that produce large quantities of

acetaldehyde could be implicated in the fruity and/or fermented flavor defect. Bills et al. (18)have demonstrated that strains of S. lactis and S. diacetilactis possess alcohol dehydrogenase activity which can effect reduction of available acetaldehyde to ethanol. The ethanol produced could then combine with free fatty acids to form fruity-like ethyl esters. In this investigation no correlation was established between the concentration of acetaldehyde in cheese curd or ripened cheese and the fruity/fermented off-flavor.

Quantification of Diacetyl in Cheese

Determination of diacetyl for several lots of experimental cheeses was performed in an attempt to assess the role of this aroma compound in the fruity flavor defect. Reference to Table 8 indicates that several of the defective cheeses exhibited a diacetyl-like flavor. The work of Calbert and Price (25, 26), pertaining to apparently critical levels of diacetyl in Cheddar cheese, has been discussed earlier.

The data in Table 11 shows that the S. diacetilactis strain employed in Vat C produced 1.78 ppm of diacetyl in the bulk starter, whereas a relatively high concentration of 10.42 ppm was found in the curd 18 hours after milling. Vat A and Vat B also showed their peak levels of diacetyl after milling the curd (2.69 and 8.07 ppm, respectively). The level of diacetyl in Vat B remained fairly

Table 11. Relative amounts of acetaldehyde, diacetyl and volatile esters found in Lot X-15 Cheddar cheese milk and curd.

Sample No.	Sample Source	ppm		
		Acetaldehyde	Diacetyl	Volatile Esters
1	Bulk starter[a]			
	Vat A	6.25	0.038	0
	Vat B	1.88	0.50	0
	Vat C	7.50	1.78	0
2	Curd at milling			
	Vat A	0.419		
	Vat B	0.291		
	Vat C	1.00		
3	Curd out-of-press			
	Vat A	0.250	2.69	0
	Vat B	0.461	8.07	0
	Vat C	0.269	10.42	0
4	Curd at 1 week			
	Vat A	0.692	0.770	0.264
	Vat B	0.334	1.23	0.132
	Vat C	0.205	0.616	1.05
5	Curd at 3 weeks			
	Vat A	0.222	0.365	0.480
	Vat B	0.370	1.31	0.480
	Vat C	0.371	0.584	0.667
6	Curd at 8 weeks			
	Vat A	0.147	0.817	0
	Vat B	0.392	1.09	0
	Vat C	0.294	0.654	0.50

a Refer to Table 3 for component species used in each vat of cheese.

constant throughout the analysis period, while the concentration in the other two vats declined.

There are few references in the literature pertaining to the diacetyl content of ripening Cheddar cheese (25, 26, 42). Mabbit (122) has stated that a thorough study of citrate fermentation in ripening Cheddar cheese is needed. This view is reinforced by the work of Calbert and Price (26) which showed that the diacetyl content of Cheddar cheese declines during ripening as the result of some unknown process. Relatively low concentrations of diacetyl (less than 0.5 ppm) were found in good quality cheese. Davies et al. (40) studied the effect of diacetyl added to cheese curd. Diacetyl added at the level of 1.7 ppm resulted in a definite "stinging" effect on the tongue of the tasters. Davis (42) proposed that changes in the oxidation-reduction potential of ripening Cheddar cheese may cause diacetyl to undergo changes.

Quantification of Volatile Esters in Cheese

Bills et al. (19) reported the isolation and identification of compounds which were believed to be responsible for the fruity flavor defect of Cheddar cheese. Certain volatile esters, primarily ethyl butyrate and ethyl hexanoate, were implicated by GLC analysis and positively identified by mass spectrometry.

An attempt was made to employ the hydroxamic acid method of

Lindsay (119, p. 87) for the determination of volatile esters in young cheese. Despite the doubtful sensitivity of the method for measuring esters in cheese, the recovery rate for ethyl acetate (added to cheese) was 80 percent.

Table 11 indicates that only very low concentrations of volatile esters were detected after the elapse of one week. Only Vat C showed the presence of any color development (approximately 0.50 ppm of volatile esters) after eight weeks ripening. The concentrations of volatile esters measured by this method may not reflect reliable results due to the limited sensitivity of the procedure. Another limitation is the fact that the recovery rate for the procedure was based on the recovery of added ethyl acetate, rather than the fruity esters (ethyl butyrate and ethyl hexanoate)(19). It is rather doubtful that the hydroxamic acid method is a useful analytical tool for determining volatile esters in Cheddar cheese.

Flavor Evaluation of Cheeses

The marketing practices of the cheese industry generally emphasize the early utilization of off-flavored Cheddar cheese, including that with a fruity and/or fermented flavor defect. Experienced cheese graders routinely examine each lot of cheese after 60 to 90 days of ripening to determine the disposition of each lot. High quality cheese (above 37.0-A.D.S.A. flavor score or

91.0-total score) are usually held for further flavor development by extending the curing period (142, p. 176). It was deemed pertinent to this study to try to assess the relative flavor preference for several typical off-flavors frequently found in commercial Cheddar cheese, especially the fruity and fermented flavor defect.

Ten samples of commercial and experimental Cheddar cheese were selected and evaluated by three experienced cheese judges to establish a set of off-flavor samples and a reference sample for presentation to a student flavor panel. The A.D.S.A. flavor scores and flavor criticisms and the flavor preference panel scores are presented in Table 12. A good quality medium-aged cheese (sample 1) was included in each test and considered as the reference sample on the basis of its possessing a typical Cheddar flavor (flat or slight lack of full Cheddar flavor). Sample 3, considered to have a slight fruity/fermented off-flavor by the three judges, did not score significantly different from the reference sample when rated by the student panel (test I, Table 12). However, a slightly acid cheese (sample 6), assigned a 39.0-flavor score by the three judges, received a score which was significantly different from the control cheese. Presumably, the panel members preferred the cheese with a slight fruity/fermented flavor (sample 3) and the cheese with an acid-unclean flavor (sample 4) to the cheese with a distinct acid taste (sample 6).

Table 12. Flavor evaluation of ten commercial and experimental Cheddar cheese samples by A.D.S.A. score and flavor preference panel score.

Sample	Flavor	A.D.S.A. Flavor Score	Reference Preference-Score[a] I	II	III	Cheese Source
1	Flat (Slight lack of Cheddar flavor)	38.5	6.49	6.52	6.36	Control cheese[b] medium-aged
2	Acid, unclean	36.5		6.54		High acid
3	Slight fermented/fruity	37.5	6.19			Commercial S. lactis[b] culture (aged 3 months)
4	Acid, unclean	36.0	6.32			High acid
5	Fermented, unclean	34.0		4.53*		S. lactis ML-3P[b] fruity culture (aged 9 months)
6	Slight acid	39.0	6.00*			Good commercial medium aged
7	Slight flat	39.0		6.51		Good commercial aged
8	Slight acid, very slight whey taint	38.5			6.48	Raw milk, medium aged
9	Acid, slight bitter	37.5			5.99*	Raw milk, aged
10	Bitter, slight rancid	36.5			4.83*	Slight rancid, aged
Number of judgements			179	174	318	
Least significant difference (LSD)*			0.42	0.42	0.28	

a Based on a hedonic scale of 1 (dislike extremely) to 9 (like extremely). b Experimental cheese.
* Significantly different at 5 percent level.

Test II (Table 12) compared an acid-unclean flavored cheese (sample 2), a slightly flat flavored cheese (sample 7) and a six month-old fermented-unclean flavored experimental cheese (sample 5) with a reference sample. The hedonic score of 4.53 for the fermented-unclean cheese sample was the lowest value recorded for any sample in the flavor panel evaluation. In test III, an acid, slightly bitter cheese (sample 9) and a bitter, slightly rancid cheese (sample 10) scored significantly lower than the control cheese. Sample 8, judged to have a slight acid, slight whey taint off-flavor by the judges, scored higher than the reference sample (6.48 vs. 6.36). It is interesting to note from this flavor evaluation study that (1) the cheese with a fermented-unclean flavor scored lower than the cheese with a slight rancid flavor, and (2) the hedonic scale values for the slight fruity/fermented sample and the cheese with a slight whey taint defect compared favorably with the reference cheese. This tends to substantiate the soundness of the cheese industry practice of marketing Cheddar cheese with fruity and/or fermented or whey taint off-flavors at an early date. This limits the occurrence of higher intensity fruity, fermented and/or unclean flavors that frequently develop in matured Cheddar cheese.

SUMMARY AND CONCLUSIONS

Selected strains of several species of lactic streptococci were subjected to various taxonomic tests, proteolytic activity studies and quantification procedures for acetaldehyde and diacetyl in an attempt to obtain data which could be useful in predicting the tendency for certain cheese cultures to develop the fruity and/or fermented flavor defect in Cheddar cheese. Control cultures and certain cheese-starter cultures, which had been implicated in earlier studies as the cause of fruity flavored Cheddar cheese, were used to manufacture 54 vats of experimental Cheddar cheese. Organoleptic analysis of the experimental cheeses indicated that the type and intensity of off-flavors in resultant cheeses was a function of the species and/or strain of lactic streptococci employed. Variation of the cheesemaking conditions was briefly studied for any possible effect on the development of the flavor defect.

Microbiological and chemical analyses were performed on the experimental cheeses to follow microbial population patterns and the formation of acetaldehyde, diacetyl and volatile esters. Existing quantification procedures for these flavor compounds were modified and adapted for analysis of fresh cheese curd and ripened cheese.

Ten commercial and experimental Cheddar cheese samples were evaluated by a flavor panel reference-preference test and

scored by three experienced cheese judges.

The following conclusions were drawn from the results of this investigation:

1. No correlation apparently exists between the milk protein hydrolytic activity of the cheese culture and the fruity/fermented flavor defect of Cheddar cheese.
2. The specific lactic streptococcus strain and/or species used as a cheese culture affected the flavor score and type of flavor in resultant cheeses, independent of the temperature of heat treatment or hydrogen peroxide-catalase treatment of cheese milk.
3. No relationship was found between the use of cheese cultures consisting of S. diacetilactis strains and the development of the fruity and/or fermented off-flavor in cheese.
4. Each vat of experimental cheese which developed the fruity and/or fermented flavor defect contained at least one or more strains of S. lactis in the starter culture. S. lactis strain ML-3P and other S. lactis strains caused the development of very intense fruity or fermented flavors in the resultant cheese within 90 days.
5. Existing procedures for the quantification of diacetyl, acetaldehyde and volatile esters were modified and adapted for analysis of cheese.

6. The factors affecting the recovery rate for acetaldehyde in cheese curd or ripened cheese were the method of slurry preparation, type of diluent and the pH.
7. A sample of fermented-unclean flavored experimental Cheddar cheese received the lowest reference-preference score in a flavor panel test, whereas a slight fruity/fermented cheese sample scored favorably with a reference sample.

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APPENDIX

Chart 1. Oregon State University Dairy Products Laboratory cheese-make sheet.

Lot Number X-6 Date July 30, 1963

	Vat A	Vat B	Vat C
Source of Starter	Culture A	Culture B	Culture C
Lbs. of Milk	850	800	800
Percent Milkfat	3.95	3.95	3.95
Lbs. of Milkfat	33.6	31.6	31.6
Lbs. Starter(@ 1 Percent)	8.5	8.0	8.0
Rennet (@ 45 ml/1000 lbs)	38.3	36.0	36.0
Cheese Color (@ 45 ml/1000 lbs)	38.3	36.0	36.0
Salt (@ 2.5 lbs/1000 lbs)	2.1	2.0	2.0
Size of Curd Knife	3/8"	3/8"	3/8"
Time			
Starter Added	11:15	11:30	11:45
Rennet Added	12:20	12:35	12:50
Cutting Curd	12:55	1:10	1:30
Steam On	1:10	1:25	1:50
Steam Off	1:45	2:00	2:25
Whey Drained	3:30	3:15	3:45
Packing	4:05	3:35	4:10
Milling	7:35	5:35	6:35
Salting	7:45	5:50	6:50
Hooping	8:05	6:15	7:15
Acidity At			
Setting	0.175	0.17+	0.175
Cutting Curd	0.11	0.11+	0.115
Whey Draining	0.13+	0.145	0.145
Packing	0.165	0.225	0.215
Stacking	0.27	0.30	0.30
Milling	0.45	0.59	0.59
Temperature in degrees C			
Heat Treatment of Milk	73.0	73.0	73.0
When Starter Added	25.0	25.0	25.0
When Rennet Added	30.6	30.6	30.6
Cooked to	40.0	40.0	40.0
Curd Cooled to	36.0	36.0	36.0
Hooping	34.4	34.4	34.4
Yield of Green Cheese			
Gross Weight (lbs)	89.25	82.0	83.50
Percent Yield	10.50	10.23	10.41

Chart 1a. Analytical data on cheese milk and resultant cheese, Lot Number X-6.

	Date July 30, 1963		
	Vat A	Vat B	Vat C
Cheese Milk			
Flavor of Milk	Feed	Feed	Feed
Milk Acidity (Percent)	0.16	0.16	0.16
Standard Plate Count	<3000	<3000	<3000
Percent Milkfat	3.95	3.95	3.95
Percent SNF	8.55	8.55	8.55
Percent Total Solids	12.50	12.50	12.50
Percent Casein	2.76	2.76	2.76
Casein/Fat Ratio	0.70	0.70	0.70
Cheese Composition			
Percent Milkfat	33.00	33.50	33.50
Percent Moisture	36.65	35.25	35.65
Percent Fat-in-Dry Matter	52.20	51.75	52.10

Chart 1b. A.D.S.A. Cheddar Cheese Score card for Lot Number X-6.

	Date November 11, 1963		
	Vat A	Vat B	Vat C
Flavor Criticism	Flat	Fermented (Unclean)	Fruity (Diacetyl-like)
Flavor Score	38.5	37.0	38.0
Body and Texture Criticism	Corky, Slits	Open, Gassy and Slits	Slight Open
Body and Texture Score	28.5	28.0	29.5
Total Score	92.0	90.0	92.5