The Effect of Psychrotrophic Bacterial Contamination on the Quality of Cheddar Cheese.

Najim Hadi Najim

Louisiana State University and Agricultural & Mechanical College

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THE EFFECT OF PSYCHROTROPHIC BACTERIAL CONTAMINATION
ON THE QUALITY OF CHEDDAR CHEESE

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Dairy Science

by
Najim Hadi Najim
B.S., University of Baghdad, 1977
M.S., Washington State University, 1981
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ABSTRACT

The main objective of this research was to determine the effect of psychrotrophic bacterial contamination and high moisture curd on the major chemical components and flavor of Cheddar cheese. Also, it was of interest to establish chemical profiles of "good" and "bad" Cheddar cheeses and to gain information which would aid in the prediction of Cheddar cheese flavor.

The research was divided into two parts. Part I concentrated on the effects of psychrotrophs in raw milk on Cheddar cheese quality. Part II examined the effects of psychrotrophic and coliform post-pasteurization contamination, high moisture curd and low activity starter on Cheddar cheese quality. After processing, Cheddar cheese was stored at 7°C and sampled at 0, 5, 30, 60 and 180 d. Analyses performed included: sensory evaluation, yield, microbiological profile, proteolytic activity, gas chromatography with headspace sampling (GCHS) and high performance liquid chromatography (HPLC).

Data revealed that the control cheese had significantly superior flavor and body/texture scores than all treated cheeses. Yield of the control cheese was significantly higher than cheese made from psychrotrophic-treated raw milk. Statistically higher levels of
citrate, acetone, 2-butanone, proteolytic activity, lactate, ethanol, propanol, formate and propionate and lower levels of acetate, 2-pentanone, pyruvate, pH and salt were noted in "bad" Cheddar cheese as opposed to "good" Cheddar cheese. All microbial counts tended to decrease while the proteolytic activity tended to increase with storage time. HPLC analysis indicated significant increases of citric, pyruvic, lactic, formic, acetic and propionic acids with aging of the cheese. Only orotic acid decreased with aging. GCHS values indicated increases of acetone, 2-butanone and ethanol with aging; 2-pentanone decreased with aging.

Excellent Cheddar cheese had low concentration of short chain free fatty acids (FFA). Young Cheddar cheese contained low concentration of FFA while aged, desirably-flavored Cheddar cheese had intermediate concentrations of individual FFA. Aged cheeses treated with psychrotrophs were signaled by measurement of high concentrations of individual short-chain and long-chain FFA. Finally, regression equations were established which laid a foundation for further study in the area of flavor prediction.
CHAPTER I
INTRODUCTION

Milk is collected on alternate days from dairy farms in most areas of the United States. However, the decrease in number of dairy farms and dairy processing plants causes some raw milk to be transported for long distances and/or held for long periods of time before it reaches a processing plant. Once the milk reaches the processing plant, it may be stored for two or more days before it is processed. This is particularly true if the milk arrives at a processing plant on Friday, since many plants operate only on a five-day per week schedule (Cousin, 1982). Storage of milk for long periods at refrigeration temperature has resulted in new quality problems for the dairy industry. These problems are related to growth and metabolic activities of microorganisms at low temperatures. These microorganisms, which are termed psychrotrophs, are ubiquitous in nature and common contaminants of milk (Thomas, 1970; Thomas and Druce, 1969; Thomas et al., 1971; Thomas et al., 1973b). As these psychrotrophs increase in number throughout refrigerated storage, enzymes are synthesized during microbial growth in the milk. These enzymes, many of which are heat-stable, biochemically alter the milk, eventually
causing spoilage. Most psychrotrophs isolated from milk are gram negative nonspore-forming rods and are species of *Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes* and *Enterobacter* (Witter, 1961; Buchanan et al., 1974). While psychrotrophic bacteria usually do not survive pasteurization, many of the lipases and proteases they elaborate can survive. These enzymes are carried into the cheese, and, in excess, could produce bitterness and rancidity (Moskowitz, 1980). In addition, the breakdown products produced by these enzymes stimulate starter culture acid production which, in excess, produces body and flavor defects (Mikolajcik, 1979). *Pseudomonas fluorescens* 27 (P27) produced both proteases and lipases that were capable of surviving heating at 70°C for 30 min as a crude enzyme (Christen et al., 1980). Mayerhofer et al. (1973) isolated and characterized protease from *Pseudomonas fluorescens* which retained 71% of its original activity after being heated at 71.4°C for 60 min. The enzyme hydrolyzed milk protein at 4°C when protease or *Pseudomonas fluorescens* was added to milk for Cheddar cheese making, and the milk then held for 12 h at 4°C prior to manufacture, flavor scores were significantly lower than those of control samples; bitterness occurred most frequently in cheese made with *Pseudomonas fluorescens*.

After storage for 8 d, tyrosine equivalent (TE) had increased 88.4% in milk stored at 4°C compared to 222% in
milk stored at 7°C. The increase in TE was always associated with an increase in the psychrotrophic count (Yan et al., 1983). The highest incidence of lipolytic activity among the psychrotrophic gram negative flora of commercial raw milk was found in strains of *Pseudomonas fluorescens* and *Pseudomonas fragi*. The above two species tested further had lipases which retained 20-25% of their activity even when heated at 100°C for 10 min (Law, Sharp and Chapman, 1976). The presence of pseudomonad lipase, carried over from stored raw milk into cheese, is a well-established source of off-flavor and has never been cited as a source of improvement even when progressive ripening was studied (Law, Sharp and Chapman, 1976; Cousin and Marth, 1977a; Cogan, 1976).

Partially purified phospholipase C from *Pseudomonas fluorescens* hydrolyzed phospholipids which were adsorbed on fat globules in a model milk composed of butter oil emulsified with crude soy lecithin. Modification of the membrane by this enzyme enhanced lipolysis when lipase was introduced to the emulsion contained in a Bio-fiber breaker-type dialysis system (Chrisope and Marshall, 1976).

The contaminating flora of cheese (*E. coli* and *P. fluorescens*), which during processing of raw milk is normally characterized by the raw milk flora of the cheese milk, significantly influences the cheese
ripening, especially the proteolysis. Of main concern is the degradation of $\beta$ casein (Gallmann et al., 1982).

Nelson and Marshall (1977) reported that yield of Cheddar cheese was reduced when milk was inoculated with an Enterobacter spp ($2 \times 10^5$/ml) and stored at $5^\circ$C for 48 h. Psychrotrophs have been reported to have proteolytic and lipolytic activity and to produce undesirable breakdown products soluble in the whey that result in lower cheese yield (Cousin and Marth, 1977a,b; Law, Sharp and Chapman, 1976). Yield reduction resulted from both lipid and protein degradation. Fat losses were estimated from whey fat content; and protein losses were observed from increased non-protein nitrogen and whey nitrogen values (Hicks et al., 1982; Swart et al., 1983). Yan et al. (1983) and Larry (1984) reported that proteases elaborated by psychrotrophic bacteria are capable of damaging the casein micelle and consequently reducing Cheddar cheese yield.

According to Elliker et al. (1964), the most important source of psychrotrophic bacteria in pasteurized milk is contaminated equipment, i.e. improperly or inadequately cleaned equipment, between the pasteurizer and the final container. Elliker (1964) and Buchanan et al. (1974) listed those genera of bacteria most frequently involved in keeping quality problems in pasteurized milk as Pseudomonas and Enterobacter. The
presence of these organisms in pasteurized milk is usually attributed to post-pasteurization contamination (American Public Health Association, 1978). Bodyfelt (1980) stated that 75 to 80% of the keeping quality problems of pasteurized milk in the United States are a direct result of post-pasteurization contamination with psychrotrophs. The presence of psychrotrophic organisms in pasteurized milk is usually attributed to post-pasteurization contamination (Witter, 1961; American Public Health Association, 1978). More recently, Wang and Frank (1981) investigated survival of psychrotrophic bacteria in commercial buttermilk and reported numbers of psychrotrophs as high as 80,000/ml in the finished products.

Although the quality of Cheddar cheese can be attributed to many factors in processing, this research centers on the effect of psychrotrophic contamination of raw and pasteurized milk and high moisture curd on Cheddar cheese quality.

Marsili et al. (1981) introduced a fast and accurate procedure for measuring organic chemicals in Cheddar cheese. This procedure utilizes high-performance liquid chromatography (HPLC) for the analysis of organic acids and headspace gas chromatography (HSGC) for the analysis of volatile organics such as acetone, 2-butanone, ethanol, 2-pentanone and propanol. The advantages of
this procedure over colorimetric methods commonly used include simplicity, less chemical interference and the capability of quantitating many flavor compounds simultaneously. In addition, Deeth et al. (1983) introduced a fast and accurate procedure for monitoring free fatty acids (C4 to C18:3) in Cheddar cheese. The identification of the agents responsible for cheese flavor is important not only to the understanding of the processes that occur during ripening but also because it may offer a chemical means of estimating flavor development.

It was the intent of this research to utilize the methods devised by Marsili and determine differences in chemical profiles of good versus bad Cheddar cheese. This research was divided into two major parts. The first part was designed to examine the effects of psychrotrophs in raw milk on Cheddar cheese quality. The second part examined the effects of post-pasteurization contamination and high moisture curd on Cheddar cheese quality.

 Flavor degradation due to proteolytic enzymes may not be accurately reflected in organic compounds examined by Marsili et al. (1981). For this reason the Hull Test (Hull, 1947) was used in this research to determine proteolysis due to psychrotrophic bacteria. Another goal of this research was to gain information which will aid in the prediction of Cheddar cheese flavor.
With this in mind, the formulated objectives of this research were as follows:

1. Prediction of ripened cheese flavor by analysis of young cheese.
2. To study the effect of highly lipolytic and proteolytic psychrotrophs on flavor development on Cheddar cheese.
3. To evaluate raw milk quality as related to Cheddar cheese acceptability and yield.
4. To monitor the microbiological pattern of Cheddar cheese during six months aging at 7°C.
5. To study the effect of post-pasteurization contamination of psychrotrophs and coliforms on the quality and yield of Cheddar cheese.
6. To evaluate the effect of high moisture cheese on bitterness and flavor profile.
7. To establish flavor profiles for Cheddar cheese rated "good" and "bad."
 CHAPTER II
REVIEW OF LITERATURE

Psychrotrophic Bacteria

In the past, microorganisms associated with cold temperature environments have often been referred to as psychrophiles. This term "psychrophile" tends to imply an optimum growth temperature below 20°C (Foster et al., 1957). Psychrotroph is a name suggested by Eddy (1960) for bacteria that grow well at low temperatures. Since most microorganisms isolated from dairy products have an optimum growth temperature above that stated for psychrophiles, it becomes necessary to distinguish between those which grow best below 20°C and those which are capable of growing and reproducing at refrigeration temperatures (American Public Health Association, 1978, hereafter referred to as APHA, 1978).

The widespread practice of storing cooled bulk raw milk before collection and often again before processing has heightened the significance of psychrotrophic bacteria in relation to the quality of dairy products (Thomas and Thomas, 1973a,b). This low temperature prevents milk deterioration from growth of lactic acid bacteria; however, the predominant microflora of the milk are psychrotrophic bacteria (Stadhouders and Mulder, 1978).
1958; Thomas, 1974; Cousin, Sharp and Law, 1977). The most recent interpretation of the term "psychrotrophs" and that which is accepted by the dairy industry today refers to "those organisms capable of appreciable growth in milk and milk products at commercial refrigeration temperatures, 2 to 7°C, irrespective of their optimum growth temperature" (APHA, 1978).

Since some starter bacteria demonstrate psychrotrophic characteristics (Wang and Frank, 1981), psychrotrophic bacteria will be referred to in this research as, those capable of growth and reproduction at refrigerated temperatures which may ultimately result in the spoilage of the finished product.

Sources and Types of Psychrotrophs in Dairy Products

The natural sources of psychrotrophic bacteria are water and soil (Witter, 1961). These bacteria are present to some extent in all raw milk supplies (Erdman and Thornton, 1951). The number of psychrotrophic bacteria in raw milk depends upon sanitary conditions prevailing during production and upon time and temperature of milk storage before processing (Olsen et al., 1955; Vedamuthu, 1978).

The presence and growth of psychrotrophic bacteria in pasteurized milk continues to be a very serious problem in the dairy industry. According to Elliker et al. (1964), the most important source of psychrotrophic
bacteria in pasteurized milk is contaminated equipment, i.e., improperly or inadequately cleaned equipment between the pasteurizer and the final container. Previous investigations, such as those of Patel and Blankenagel (1972), have shown that raw milk with minimum psychrotrophic counts of $10^7$ colony forming units (CFU) per milliliter invariably gave rise to flavor defects in pasteurized milk. Bodyfelt (1980) stated that 75 to 80% of the keeping quality problems of pasteurized milk in the United States is a direct result of post-pasteurization contamination.

Due to the extended refrigerated storage of raw and pasteurized milk, as well as other dairy products, the presence of psychrotrophic bacteria becomes increasingly important. These bacteria are capable of producing off-flavors such as fruity, stale, bitter, putrid, unclean, sour and rancid, as well as physical defects (Elliker et al., 1964; APHA, 1978).

Most psychrotrophs isolated from milk are gram negative nonspore-forming rods and are species of Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Escherichia and Aerobacter (Enterobacter) (Witter, 1961). Elliker (1964) and Buchanan et al. (1974) list those genera of bacteria most frequently involved in keeping quality problems in pasteurized milk as Pseudomonas, Anchromobacteriam, Alcaligenes, Proteus, Escherichia and
Aerobacter (Enterobacter). The presence of these organisms in pasteurized milk is usually attributed to post-pasteurization contamination (Witter, 1961; Elliker, 1964; APHA, 1978).

In addition to gram negative psychrotrophs, raw and pasteurized milk contain some gram positive, spore-forming thermoduric rods belonging to the genera Bacillus and, more rarely Clostridium (Shehata and Collins, 1972; Washam, Olson and Vedamuthu, 1977). The ability to form spores enables them to survive heat treatments and reproduce at refrigeration temperature (Mikolajcik, 1978). The growth of psychrotrophs in stored raw milk often affects the efficiency with which the milk can be utilized in terms of (1) quality and flavor of milk products; (2) shelf-life; (3) cheese yield (Law et al., 1979b). The importance of psychrotrophic bacteria has greatly increased with extended storage of raw and pasteurized milk and other dairy products. These bacteria are reported to cause a variety of off-flavors, including fruity, stale, bitter, putrid, rancid, or yeasty flavors. Some produce sourness, as well as physical defects (Thomas, 1948; Foster et al., 1957; Hammer, 1957; Thomas, 1969; Vedamuthu et al., 1978; Mikolajcik, 1978; Bodyfelt, 1980; Collins, 1981). Excessive growth of gram negative bacteria in milk before processing may have a stimulatory effect on subsequent
growth of psychrotrophic sporeformers (Overcast and Atmaram, 1974).

Production of Protease by Psychrotrophic Bacteria

As early as 1957, Camp and Van der Zant demonstrated the ability of a cell free extract from *Pseudomonas putrefaciens* to hydrolyze peptides containing the amino acid tyrosine. Other investigators reported the action of proteolytic enzymes in a variety of food products (Peterson and Gunderson, 1960a and 1960b).

Fryer et al. (1966) produced three different types of cheese: (1) In one case *Streptococcus cremoris* or *Streptococcus lactis* was used as the inoculum along with a predetermined mixed flora, called reference floras, which were isolated by Rieter et al. (1965) and Fryer et al. (1966). This flora consisted of adventitious organisms (gram negative rods) introduced during cheese making as contaminants. The cheese so made developed intense Cheddar flavor in a shorter time, but the flavor quality was variable and often off-flavors predominated. (2) In another vat, cheese was made in the absence of the reference floras, and the starter consisted of a single strain inoculum of *Streptococcus lactis* or *Streptococcus cremoris*. Such cheese had consistent flavor attributes, and the off-flavors resulting from the starter were reproducible, indicating these flavor defects were
characteristic of the strain of culture used in making the cheese.

Schormuller (1968) has demonstrated that proteases, peptidases, transaminases, decarboxylases and phosphatases are involved in the flavor and texture development of both soft and hard types of cheeses.

In more recent years, Patel and Blankenagel (1972) reported flavor degradation during storage of pasteurized milk and related these results to heat resistant proteolytic enzymes present in the raw milk supply. These workers stated that psychrotrophs at levels greater than 1,000,000/ml in the milk, resulted in a bitter flavor during storage of the finished product even though numbers after processing were low.

Mayerhofer et al. (1973) isolated and characterized protease from *Pseudomonas fluorescens* which retained 71% of its original activity after being heated at 71.4°C for 60 min. The enzyme hydrolyzed milk protein at 4°C. When protease or *Pseudomonas fluorescens* was added to milk for Cheddar cheese making, and the milk then held for 12 h at 4°C prior to manufacture, flavor scores were significantly lower than those of control samples; bitterness occurred most frequently in cheese made with *Pseudomonas fluorescens*, and unclean flavor in those made with protease. Also, proteolysis was significant in Cheddar cheese made from milk containing .94 unit of enzyme per milliliter (White and Marshall, 1973).
Juffs (1974) reported that added pseudomonas proteinase did not increase bitterness in Australian Cheddar cheese. Also, he reported off-flavor formation by pseudomonas proteinases, but this work concerned enzymes added in amounts calculated to aid milk coagulation, and although no relationship was established with corresponding viable psychrotroph count in milk, it can be assumed that relatively high enzyme concentrations were involved.

Malick and Swanson (1974) later demonstrated the ability of proteolytic enzymes produced by some psychrotrophic bacteria to withstand sterilization at 135°C for 3.5 min and still hydrolyze milk protein. Adams, Barach and Speck (1975) and Driessen (1976) reported that added heat resistant pseudomonas proteases caused rapid spoiling (by clearing or coagulation) of sterile milk with the development of bitter flavor. White (1971) reported the number of psychrotrophs necessary to produce one unit of enzyme activity as $1.5 \times 10^7$/ml. Adams et al. (1975) revealed numbers as low as 1,000/ml which were capable of producing 20 units of protease/ml. In the same study, it was noted that 70-90% of the raw milk samples examined contained psychrotrophs capable of producing heat resistant proteases.

Chapman et al. (1976) found that differences in time needed for Cheddar cheese manufacture depended on how milk was stored before it was made into cheese.
Processing time was decreased and a firmer curd resulted when Cheddar cheese was made from milk cultured with psychrotrophic bacteria rather than control milk. One factor related to curd firmness could be the initial pH; that of control cheeses was 6.70 compared to 6.55 to 6.60 for cheeses from treated milk (Cousin and Marth, 1977a). Cousin and Marth (1977b) also reported shorter manufacturing times for Cheddar cheese made from stored milks which had contained up to $10^6$ CFU/ml of caseinolytic psychrotrophs. They also reported that psychrotrophic bacteria caused changes in stability of milk to coagulation by rennet or heat because all pasteurized and inoculated milks clotted in substantially less time than did pasteurized control milk when rennet was added. Kappa-casein degrading enzyme (proteinases) produced during the growth of a psychrotrophic strain of *Pseudomonas fluorescens* can produce gelation of stored UHT-sterilized milk (Law, Andrews and Sharpe, 1977).

The degradation of proteins contributes to a softening of the cheese, thereby altering texture. Proteins are degraded by microbial proteases to yield peptides and amino acids. These amino acids can undergo a variety of changes, such as side chain alterations, decarboxylation, transmission and oxidative deamination to alpha-keto acids (Arun and Shahani, 1978).

Proteolysis in milk increased with an increase in storage time. Increases in tyrosine equivalent (TE) were
greater in milk stored at 7°C. Marked increases in TE were observed after 4 d of storage at 7°C and after 6 d of storage at 4°C. After storage for 8 d, TE had increased 88.4% in milk stored at 4°C compared to 222% in milk stored at 7°C. The increase in TE was always associated with an increase in the psychrotrophic count. The increase in TE occurred 2 to 4 d after the psychrotrophic count exceeded $10^6$ CFU/ml (Yan et al., 1983).

**Production of Lipase by Psychrotrophic Bacteria**

Several workers (e.g., Pinheiro, Liska and Parmelee, 1965; Koshonti and Sjostrom, 1970; Driessen and Stadhouders, 1971) have shown that extracellular lipases from raw milk psychrotrophic bacteria (Pseudomonas and Alcaligenes spp) are resistant to pasteurization temperatures which kill the bacteria themselves. Such enzymes subsequently caused excessive lipolysis and rancidity in cream, butter and cheese. Ohren and Tuckey (1969) observed that high levels of free fatty acids (FFA) developed during maturation in cheeses made from heat treated milk if levels of organisms were high in the raw milk, indicating that surviving bacterial lipases were also active in cheese. Similarly, Sharpe (1972) showed that although reference floras (Reiter, Fryer and Sharp, 1965; Fryer, Sharp and Reiter, 1966) including psychrotrophic gram negative rods (GNR), inoculated into
low count milks and stored at low temperatures were eliminated by pasteurization, their lipolytic activity survived the process and gave rise to rancid flavored Cheddar cheese containing high levels of free butyric and higher fatty acids.

The highest incidence of lipolytic activity among the psychrotrophic gram negative flora of commercial raw milk was found in strains of *Pseudomonas fluorescens* and *Pseudomonas fragi*. The above two species had lipases which retained 20-25% of their activity even when heated at 100°C for 10 min (Law et al., 1976). Cheeses made from milks in which mixed strains of lipolytic gram negative rods (GNR) had been allowed to multiply to greater than 10⁷ CFU/ml became rancid after 4 mo even though the GNR had been killed by pasteurization. A single strain of *Pseudomonas fluorescens* (ARII) produced the same effect after only 2 mo under similar conditions. The rancidity was characterized by a soapy off-flavor in cheeses containing free fatty acid concentrations from three to ten times higher than those in control cheeses made from stored milks with low GNR counts. Strong rancidity could be reproduced by adding the culture supernatant of a pre-grown lipolytic strain, but not the washed cells, and pasteurizing it immediately before cheese-making, demonstrating the extracellular nature of the rancidity including lipases (Law, Sharp and Chapman, 1976).
Psychrotrophic bacteria growing in refrigerated raw milk can produce heat-resistant enzymes which subsequently affect dairy product quality adversely. For example, lipases may cause rancidity in Cheddar cheese (Law, Sharpe and Chapman, 1976). While psychrotrophic organisms usually do not survive pasteurization, the lipases and proteases they elaborate do survive. These enzymes are carried into the cheese and, in excess, could produce bitterness and rancidity (Moskowitz, 1980). In addition, the breakdown products produced by these enzymes stimulate starter culture acid production which, in excess, produces body and flavor defects (Mikolajcik, 1979).

Andersson (1981) reported that the thermostable lipase of *Pseudomonas fluorescens* SIK WI had a marked effect on the flavor of ultra-pasteurized milk. Samples with .3 lipase unit per milliliter were perceived as "rancid" after 5 to 8 d of storage at 8°C. *Pseudomonas fluorescens* 27 (P27) produced both protease and lipase that were capable of surviving heating at 70°C for 30 min as a crude enzyme (Christen et al., 1980). Lipase and protease were produced simultaneously by *Pseudomonas fluorescens* 27 (P27) in three types of broth and on a dialysis membrane resting on semisolid media (Christen et al., 1984).
Production of Phospholipase by Psychrotrophic Bacteria

Milk is comprised of a complex mixture of fat, protein, sugars, and inorganic salts in states of emulsion, colloidal dispersion, and solution. The fat exists as minute globules emulsified by a coating or "membrane." This membrane of protein and phospholipid serves as a physical barrier to prevent agglomeration of the globules during the agitation, and it also may offer protection against lipolysis. Its removal may allow easy accessibility of lipases from milk or microorganisms to the glycerides of the fat globule (Law et al., 1973 and Mabbitt, 1981).

Involvement of milk fat in the flavor of Cheddar cheese is apparent from observations that cheese made with low fat milk or skim milk fails to develop the characteristic flavor (Ohren and Tuckey, 1969). The fat globule membrane (FGM) is an additional source of lipids (in particular, phospholipids), and its possible contribution to cheese flavor has not been considered previously, although recent reports have dealt with isolation of phospholipids from cheese (Umemoto and Sato, 1970; Nakanishi and Kaya, 1971). Phospholipase C hydrolyzed about 90% of the lipid phosphorus of both low and high-density lipoprotein fractions of milk (O'Mahony, 1972).

Cheddar cheese made with skim milk and butteroil (without buttermilk) homogenized at 422 $10^3$kg/m$^2$ were
rancid after 6 mo ripening and contained very high amounts of free fatty acids. Phospholipid in the cheeses decreased by 50 to 70% during the 6 mo ripening. The breakdown of phospholipids during ripening of Cheddar cheeses was attributed to phospholipases produced by the starter bacteria (Law et al., 1973). However, Chrisope and Marshall (1976) discovered that none of the lactic cultures examined displayed phospholipase activity on lecithin agar after 15 d incubation.

Partially purified phospholipase C from Pseudomonas fluorescens hydrolyzed phospholipids adsorbed to fat globules in model milk composed of butteroil emulsified with crude soy lecithin. Modification of the membrane by this enzyme enhanced lipolysis when steapsin (lipase) was introduced to the emulsion contained in a Bio-fiber breaker-type dialysis system. Following an initial period of induction, the velocity of lipolysis in the model emulsion was greater in the presence of phospholipase C than in its absence. Activity of milk lipase in raw milk was enhanced also. Titers of free fatty acids increased at greater rates in raw milks incubated at 30°C with phospholipase C than without it (Chrisope and Marshall, 1976).

Doi and Nojima (1971) experienced approximately 45% loss in activity after heating phospholipase C from Pseudomonas fluorescens to 60°C for 5 min in buffer
without glycerol. However, Chrisope's and Marshall's crude enzyme preparation (60% ammonium sulfate precipitate in glycerinated buffer) from Pseudomonas fluorescens 178 produced typical phospholipase C reaction in lecithin agar after being heated at 60°C for 20 min.

Bacteria which produced phospholipase C were isolated from 13 of 34 fresh and 15 of 35 spoiled samples of homogenized milk stored at 4 to 6°C. No single off-flavor was assigned consistently to samples with phospholipase producers, but 75% of them were bitter. Pseudomonads constituted 62% of the isolates. Other phospholipase C-producing genera and their numbers were Acinetobacter, two; Alcaligenes, three; and Flavobacterium, two (Fox et al., 1976). These results and the findings of Chrisope et al. (1976) that psychrotrophic bacteria of milk are major producers of phospholipase C give further emphasis to the need to minimize numbers of psychrotrophic bacteria in milk (Fox, 1976).

Lecithin agar was developed by Chrisope et al. (1976) on which a culture of Pseudomonas fluorescens 178 produced an opaque zone, suggesting phospholipase C activity (formation of water-insoluble diglyceride). Strain 157 of an unknown species of Pseudomonas produced a clear zone, which was attributed to activity of phospholipase A₁ or A₂ (formation of water-soluble lysolecithin).
Factors Affecting Cheddar Cheese Yield

A direct linear relationship has been demonstrated between amount of fat and casein in milk and yield of Cheddar cheese (Price, 1952). Similar results have been observed during manufacturing of cottage cheese (Mickelsen, 1974). Irvine et al. (1969) reported that Cheddar cheese yields tended to be lower because of high proteolytic activity of a protease complex from a mutated strain of Bacillus subtilis as a rennet substitute and such cheeses were of excellent quality with no bitterness. Cheese yields were lower with microbial rennets produced by Mucor pusillus lindt and Bacillus polymyxa than that from calf rennet (Kikuchi and Toyoda, 1970).

The Canadian study also indicated that cheese yield varied directly with seasonal deviations in amounts of fat plus protein in milk (Irvine, 1974).

Modern cheesemaking plants can operate efficiently only if an adequate supply of milk is available at the start of the cheesemaking process. Thus, it has become a common practice to store incoming raw milk for several days at low temperatures before using it for the manufacture of cheese (Chapman et al., 1976). The effect of low temperature storage on milk quality and on cheesemaking has concerned the dairy industry in recent years. Research has indicated that growth of proteolytic
psychrotrophs during low temperature storage may decrease milk quality, which results in a decrease in cheese yield due to the degradation of milk proteins and increased loss of nonprotein nitrogen in whey (Aylward et al., 1980).

Factors affecting cheese yield are a major concern of those in the cheese industry. Current techniques of handling raw milk often resulted in high psychrotrophic counts before cheese manufacture. Psychrotrophs have been reported to have proteolytic and lipolytic activity and to produce undesirable breakdown products in milk (Adams et al., 1976; DeBeukelaer et al., 1977; Law, Sharp and Chapman, 1976; Nelson and Marshall, 1977). Several researchers (Cousin and Marth, 1977a,b,c; Law, Sharp and Chapman, 1976; Nelson and Marshall, 1977) have stated that breakdown products soluble in the whey result in lower cheese yield. Allauddin et al. (1976) reported that yield of cheese was reduced when large numbers of psychrotrophs were present in milk. This finding was supported by Cousin and Marth (1977a,b,c) who observed a considerable increase in total nitrogen in whey from milk inoculated with psychrotrophs.

The work of Feuillat et al. (1976) has already been cited in relation to protein breakdown in refrigerated milk. These authors showed that excessive amounts of $N$ were lost into the whey of soft cheese made with heat-
treated high-count milks (approximately $10^6$ psychrotrophs per milliliter). The projected loss of cheese yield represented by the whey N losses was about 5%, a figure of considerable economic significance.

Inoculation of milk with $10^5$ proteolytic psychrotrophs per milliliter followed by incubation at 20°C for 48 h had no significant effect on curd yield with 8 of 9 test cultures. With an Enterobacter species, 38% less curd was obtained in the presence of $10^9$ organisms per milliliter; yields also decreased when an initial count of this bacterium were $2 \times 10^5$/ml and storage at 5°C for 48 h (Nelson and Marshall, 1977).

Yates and Elliot (1977) calculated that the growth of unspecified proteolytic psychrotrophic bacteria in whole milk resulted in whey N losses equivalent to reductions of 2.75-4.60% in the protein available for cheese. The lower percentage losses were associated with bacterial populations of approximately $10^7$ CFU/ml, which could be encountered in stored market milk in extreme cases (assuming the majority of the psychrotrophic flora to be proteolytic), though the whey separation was again atypical since the casein was acid precipitated and rennet was not used. Cousin and Marth (1977b) reported that percentage yields of cottage cheese curds were higher than normal when made from milk previously inoculated with Pseudomonas or Flavobacterium species.
and stored at 4.4°C for 3 d, but lower if the milk were stored for 5 d.

In contrast to these findings, Law et al. (1979a) reported that proteolytic psychrotrophs growing for 72 h in raw milk at 7.5°C to levels of approximately $10^7$ CFU/ml are unlikely to cause a significant change in the yields or quality of Cheddar cheese through their proteolytic activity. None of the cheeses developed off-flavor related to excessive protein breakdown, but many became lipolytically rancid. Even the addition of known amounts of a proteinase from *Pseudomonas fluorescens* ARll (equivalent to a viable population of $1 \times 10^7$) did not bring about detectable protein breakdown during the 72 h storage period of raw milk (Law et al., 1979b).

The yield of direct-acid cheese manufactured from inoculated milk with psychrotrophic *Bacillus* and *Pseudomonas* isolates decreased as psychrotrophic inoculation level increased. Yield reduction resulted from both lipid and protein degradation, and accounted for approximately 45 and 55% of the dry matter loss, respectively. Fat losses were observed from decreased milk fat tests and increased acid degree values. Protein losses were observed from increased non-protein nitrogen and whey nitrogen values (Hicks et al., 1982).

The pH of the milk decreased .08 unit at 4°C and .15 unit at 7°C during the 8 d storage period (Yan et al.,
1983). Chapman et al. (1976) reported that the pH of milk either did not change or it decreased during storage at 5, 7.5 and 10°C for 3 d. The rate of decrease is probably related to the type of psychrotrophs present in a particular milk supply. Cousin and Marth (1977a) suggested that psychrotrophs can decrease the pH of milk during low temperature storage, thus decreasing the soluble casein. As soluble casein decreases, micellar casein would increase which may account for the slight increases observed in yield. This shift may also cause a more rapid rennet coagulation which results in a firmer curd that is less likely to fracture and cause loss of nitrogen into the whey, thus decreasing the TE concentration in the whey. A change in solubility of milk protein during the initial storage period also may account for the increased cheese yield. Ali et al. (1980) reported a dissociation of micellar casein into the soluble phase during the first 48 h storage at 4 or 7°C.

Cheddar cheese yield was not affected when grade A raw milk was stored at 4 or 7°C up to 6 and 4 d, respectively. A rapid decrease in yield was observed when bacterial counts were greater than 10^8 CFU/ml. Decrease in yield was related to increases in proteolysis of the raw milk and to increases in TCA-soluble nitrogen in whey. Both pH and proteolysis of the raw milk
influenced moisture content of cheese. Renneting time increased with increased storage time of raw milk. Majority of yield loss occurred when raw milk was held more than 6 d at 4°C or 4 d at 7°C. Loss in cheese yield was greater for milk stored at 4°C for 8 d than for milk stored at 7°C for 6 d (Yan et al., 1983).

Yan et al. (1983) reported that the moisture content of the experimental cheese curd tended to decrease during the initial 6 d raw milk storage at 4°C then moisture content increased for cheese made from milk stored 8 d at 7°C. Decreases in the moisture content of cheese appeared to be associated with decreases in the pH of raw milk during the initial 6 d of storage. Acidulation of milk closer to the isoelectric pH of casein micelles has been reported to increase the firmness of the glucono-delta-lactone (GDL) induced curd. This increased firmness may cause more water to be expelled from the coagulum because fewer open structures are formed in the casein micelles. Although the pH of the milk was still decreasing after 8 d, the moisture content increased for cheese made from milk stored 8 d. The increase was greater for milk stored at 7°C. The increase in moisture obtained for cheese made from milk stored over 6 d at 4 and 7°C indicates that beyond a certain point proteolysis rather than pH of the milk determines the moisture content of cheese curd. Raw milk held under prolonged storage may undergo excessive proteolysis which increases
the waterbinding capacity of the denatured milk proteins in the curd. The soft-bodied curd also may be caused by its high moisture content. The average moisture content of the curd obtained in this study was higher than that of commercial Cheddar cheese (45 vs. 39%).

A report from South Africa by Swart et al. (1983) indicated that Cheddar cheese yield decreased as counts of psychrotrophs in raw milk increased on storage. Cheese yield reduction was correlated with both lipid and protein degradation.

Larry (1984) reported that proteases elaborated by psychrotrophic bacteria are capable of damaging the casein micelle and consequently reducing yield of cheese.

Primary factors related to poor cheddar cheese yields were low casein content of milk, low ratio of casein to fat, and excessive mechanical breakage of cheese curd in the vats (Barbano et al., 1984).

The economic losses for the dairy industry due to decreased cheese yield are reported by Yan et al. (1983) as follows: The greatest dollar loss occurred for the cheese made from milk stored at 7°C for 8 d ($5.32/100 kg milk). Yield loss was greater for milk stored at 4°C for 8 d ($1.43/100 kg milk) than for milk stored at 7°C for 6 d ($0.74/100 kg milk). Most of the losses occurred after the milk had been stored more than 4 to 6 d.
Survival of Psychrotrophic Bacteria in Cultured Products

In recent years, several reports have implicated the detrimental effects of lactic cultures on psychrotrophs. The term "antibiosis" is used to describe these effects and has been defined as the "antagonistic association between microorganisms to the detriment of one of them" (Babel, 1977).

Juffs and Babel (1975) have studied the inhibition of psychrotrophs due to the addition of a variety of commercial lactic cultures to milk. They noted a decline in the inhibition of *Pseudomonas fluorescens* between 48 and 72 h at 7°C. From these results, they concluded that a psychrotroph might be able to overcome the inhibition associated with lactic cultures. In the same study, it was revealed that the inhibition of psychrotrophs resulted in a reduction of growth rate rather than the reduction of initial psychrotrophic populations.

Goel et al. (1971) studied the survival of coliform bacteria in a variety of cultured products. After 24 h storage at 7°C, results indicated a large decline (50% reduction) in initial numbers of *Enterobacter aerogenes* and *Escherichia coli* in yogurt, buttermilk and sour cream. Results for cottage cheese, indicated the same counts remained fairly constant for 48 h even though increases were noted in two of the samples tested.

Sadovski et al. (1980) later examined psychrotrophic growth in a cultured product. They were unable to
isolate common psychrotrophs such as *Pseudomonas*, *Alcaligenes*, *Achromobacter* and *Flavobacterium* but did isolate gram negative rods of *Klebsiella*. More recently, Wang and Frank (1981) investigated psychrotrophic bacteria in commercial buttermilk and reported numbers of psychrotrophs as high as 80,000/ml in the finished products. Of the isolates identified, 59% were species of *Pseudomonas*.

**Effect of Psychrotrophic Bacteria on Cheddar Cheese Ripening**

The acceleration of flavor in ripened Cheddar cheese has been the goal of many investigators (Forss et al., 1966; Reiter et al., 1966; Iwasaki et al., 1973). Therefore, it is desirable to develop systems that will enhance flavor development and decrease storage cost (Moskowitz, 1980). Long ripening period adds to the cost of production due to the increased labor and refrigeration requirements which in turn affects the retail price of cheese (Barraquio et al., 1982).

Gripon et al. (1977) concluded that the addition of microbial enzymes to cheese curds is a potential method to improve quality and accelerate ripening by developing higher soluble protein and free volatile fatty acids. The development of typical flavor in Cheddar cheese was accelerated by the addition of bacterial neutral proteinase obtained from *Bacillus subtilis* to the curds,
but not by the addition of fungal acid proteinase which produced a bitter defect even at low concentrations. Enzyme-treated cheeses were softer-bodied and more brittle than untreated cheeses of the same age (Law and Wigmore, 1982). Sood and Kosikowski (1979) and Law (1980) have shown that controlled additions of very small amounts of proteinases and lipases can produce strong flavor in American Cheddar cheese in a relatively short time without causing flavor defects. Kosikowski and Iwasaki (1975) added a variety of commercially available lipases and proteinases to Cheddar cheese curd using salt as a vehicle of addition. The enzyme-containing cheese had more intense flavor than control cheese, but the degree of flavor acceleration was not expressed quantitatively. They also pointed out the unresolved problem of flavor balance created by the enzymic method for accelerating Cheddar cheese ripening.

A slightly rancid flavor is said by some investigators to be a normal and desirable component in mature cheese (Kosikowski and Iwasaki, 1975). The presence of pseudomonas lipase, carried over from stored raw milk into cheese, is a well-established source of off-flavor and has never been cited as a source of improvement even when progressive ripening was studied (Law, Sharp and Chapman, 1976; Cousin and Marth, 1977; Cogan, 1977).
The addition of cultures of *Escherichia coli* to milk did not affect protein hydrolysis in cheese made from this milk, nor were the microorganisms of the cheese surface important in this respect (Stadhouders, 1960). The contaminating flora of cheese (*E. coli* and *Ps. fluorescens*), which during processing of raw milk is normally characterized by the raw milk flora of the cheese milk, significantly influences the cheese ripening, especially the proteolysis. Mainly concerned is the degradation of β-casein (Gallmann et al., 1982).

It may be expected that increasing the population of starter streptococci in cheese would accelerate the ripening process. However, high viable starter populations in cheddar curd are reported to produce less intense typical flavor and a high incidence of bitterness subsequently in cheese (Lowrie, Lawrence and Peberdy, 1974).

**Flavor Compounds in Cheddar Cheese**

Although research on cheese flavor has been going on for more than 60 years, the chemical identity of the components of the Cheddar flavor remains unknown (Reiter et al., 1966). This lack of progress was mainly a consequence of the complexity and variability of the raw material since it was not even possible to define the individual contributions made by the chemical composition of the milk, the enzymes of the milk, the rennet and
bacterial flora. The chemical composition of the milk and particularly of the milk fat varies according to breed, individual, health of the animal, lactation, feed and season (Hansen and Shorland, 1952; Jack and Smith, 1956; Keeney, 1956; Rook, 1959; Brown, Stull and Stott, 1962; Garton, 1963; Hawke, 1963; Bottman, Hoschkiss and Hammond, 1965; Stull and Brown, 1964, 1965; Storry and Rook, 1965). While the components of cheese flavor can not be defined, it is generally recognized that its development depends on three enzymatic processes: glycolysis, proteolysis and lipolysis (Reiter et al., 1966).

Mulder (1952) proposed what is now known as the Component Balance Theory. This theory suggested that Cheddar flavor was made up of a balance of flavors contributed by a number of different compounds. When the balance was upset by an excess or lack of one or more of the component compounds, atypical flavor was produced. As the cheese ripens, flavor develops as it is during the ripening process that milk proteins, fat and lactose are subjected to multiple physical, chemical and sensory changes through the action of rennet and microorganisms (Behnke, 1980).

A consideration of the literature from the last twenty-five years shows that sophisticated analytical techniques (chiefly GC and MS) have revealed the presence
of a wide range of potentially flavorful compounds in cheeses. These include the following categories: volatile and nonvolatile fatty acids, alcohols, esters, lactones, ketones, aldehydes, hydrocarbons, pyrazines, peptides, amino acids, amines and sulphur compounds (Law, 1982).

The major flavor compounds formed in Cheddar cheese from fermentation are lactic acid, diacetyl and acetic acid, while from maturation are amino acids, amines, volatile fatty acids, pentanone, hydrogen sulphide and methanethiol (Law, 1982). The subsequent development of distinctive flavors depends on a variety of factors including the moisture level, method and extent of salting, developed acidity, and complexity of the secondary microflora (Law, 1982).

The flavor of very young Cheddar cheese is similar to that of other internally-salted varieties made with mesophilic starters; it can be described as acid, slightly buttery and salty. The flavor compounds at this stage of manufacture are largely derived from the carbohydrate fermentation of the starter streptococci; these organisms are regarded by taxonomists as homofermentative with lactose as they produce mainly lactic acid. However, they do possess alternative pathways of pyruvate metabolism which are expressed in the cheese vat to a degree which allows production of
acetic acid, ethanol and acetaldehyde (Law, 1981 and 1982). The importance of these pathways was demonstrated by Czulak and coworkers (1974) who showed that pyruvate dehydrogenase activity (a key step in diverting pyruvate to the flavorful metabolites) in starter streptococci was inhibited in cheese made with milk containing high levels of polyunsaturated fat. The cheeses were low in acetate, acetaldehyde and diacetyl and had rather bland flavor.

The important role of diacetyl in Cheddar flavor aroma is supported by the analytical work of Manning and Robinson (1973) who identified it as one of eight compounds which contributed to typical aroma in low-boiling Cheddar cheese distillates.

Bacterial growth in the Cheddar cheese is limited by the conditions of acidity, salt concentration and redox potential so that full, mature flavor may take up to 12 mo to develop (Law, 1982).

None of the volatile sulphur compounds (hydrogen sulphide, carbonyl sulphide, methanethiol and dimethyl sulphide) was considered to be useful as a reliable indicator of flavor development in Cheddar cheese (Aston et al., 1983).

Importance of Glycolysis to Typical Cheddar Cheese Flavor

The starter streptococci added to the cheesemilk ferment the milk lactose rapidly during the making of the curd and this continues in the young cheese. Suzuki,
Hastings and Hart (1909) had already shown that the lactic acid content of cheese increases up to 3.5 mo of maturation, by which time most of the streptococci have died out (Dawson and Feegan, 1957; Perry, 1961). Mabbitt and Zielinska (1956) demonstrated the presence of both lactose and galactose in the expressed juice of mature cheese, and Mabbitt (1961) suggested later that these and other sugars could be released either during the breakdown of the carbohydrates associated with milk proteins or by lysis of the streptococcal cell walls after death. A carbohydrate source is thus available for the lactobacilli, which reach their highest numbers after the streptococci (Naylor and Sharpe, 1958; Johns and Cole, 1959). In addition to lactic acid, the homofermentative lactic acid bacteria produce small amounts of acetic acid, alcohol, glycerol, carbon dioxide, hydrogen sulphide, diacetyl, acetoin and 2-3-butanediol. Young Cheddar cheese has a fresh lactic aroma, often slightly buttery, probably due to diacetyl. Its taste is slightly sour and rather bland, and the salt added to the curd can still be tasted. The saltiness, as in the case of bread, is necessary as a background but it should not be obvious in a mature cheese. Excessive numbers of coli-aerogenes or other heterofermentative bacteria are likely to produce strong off flavors in the young cheese, and it is
possible to detect differences in flavor between young cheese made with pure strains of homofermentative streptococci and those made with commercial mixed strain starters. Experimental cheeses made with *Streptococcus cremoris* under aseptic conditions have been found, for instance, to contain appreciably less acetic acid and free fatty acids of carbon chain length \(>4\) than cheeses made with a commercial starter (Reiter et al., 1966).

**Importance of Free Fatty Acids (FFA) to Typical Cheddar Cheese Aroma/Flavor**

The free fatty acids in cheese are derived from two major sources: (1) breakdown of the fat by lipolysis and (2) metabolism of carbohydrates and amino acids by bacteria. The bulk of evidence indicates that lipolysis is the principal contributor of free fatty acids of chain length \(C_4\) or greater (Foda et al., 1974; Dulley and Grieve, 1974). Milk lipases have been shown to be more active than starter lipases in Cheddar (Reiter and Sharpe, 1971). They seem to hydrolyze the fat selectively and to be able to attack triglycerides, while lactic streptococci lipases seem to be active mainly on mono- and diglycerides (Stadhouders and Veringa, 1973).

Patton (1963) claimed that volatile fatty acids \((C_2- C_8)\) were the "backbone" of Cheddar aroma because blocking agents for carboxylic functional groups impaired the aroma of cheese fat distillates. However, Manning and
Price (1977) argued that side-reactions could have occurred in these experiments which would leave the results open to different interpretations. These investigators showed that the removal of volatile fatty acids from Cheddar cheese head space did not affect its aroma at all and concluded that these acids were only important in the background taste of the cheese. Further evidence against the importance of fatty acids comes from the analysis of New Zealand Cheddars of which 23 of 41 contained no acids higher than C₄ (Lawrence, 1967).

Nakae and Elliott (1965) demonstrated that the FFA from acetic to caproic were produced from casein hydrolysates by lactic acid bacteria. Ohren and Tuckey (1969) found that Cheddar cheese containing certain levels of acetate and free fatty acids and whose acetate to fatty acid ratio was between specified limits, developed good flavor after 180 d ripening. This observation could not be confirmed by Reiter and Sharpe (1971). Acetic acid can be produced from citrate, lactose and amino acids (Fryer, 1969). Farrer and Weeks (1970) attributed Cheddar cheese flavor to one or more of the following compounds: acetic, butyric, caproic, capric acids, methionial, 3-mercaptopropionic acid. The free fatty acids butyric, caproic, caprylic and capric were included in a patent specification submitted by Henning (1970) which was claimed to impart Cheddar cheese
flavor to foodstuffs. Singh and Krostoffersen (1970) and Forss (1971) concluded that the free fatty acids were important to Cheddar cheese flavor, while Singh and Kristoffersen (1971), Gray and Walker (1972), Law, Castanon and Sharpe (1976), and Law and Sharpe (1977) found no relationship.

The normal levels of fatty acids found in cheese (approximately 500 ppm) represent amounts well above typical flavor and aroma thresholds, ranging from .3-100 ppm (Baldwin et al., 1973) so that they would be expected to contribute to the overall organoleptic qualities of cheddar cheese. However, claims that fatty acids are important because low-fat or fat-free cheeses do not develop flavor (Ohren and Tuckey, 1969) are oversimplistic since deviations from an optimum fat percentage in the product alters its characteristics markedly and may have indirect effects on flavor retention and perception. Fat-free "Cheddar" is so unlike normal Cheddar as to be irrelevant to the discussion. It has been well documented that skim milk cheese has considerably lower levels of free fatty acids than normal cheese (Ohren and Tuckey, 1956; Deane, 1972; Deane and Dolan, 1973; Foda et al., 1974; Dulley and Grieve, 1974).

Amounts of volatile fatty acids other than acetic increase during Cheddar cheese maturation due to the weak
esterase and lipase activities of the milk flora and the starter bacteria (Stadhouders and Veringa, 1973). Although these volatile fatty acids are included in many synthetic cheese flavor formulations (Henning, 1970 and Ney et al., 1972), evidence for their contribution to typical Cheddar cheese aroma/flavor is equivocal and contradictory. Acetic acid has a less clear function since its concentration can vary considerably between cheeses without concomitant variability in the quality or intensity of typical flavor (Law, Castanon and Sharpe, 1976). It probably adds to the sharp mouthfeel of cheese conferred by the high lactic acid concentration, but overproduction of acetic acid can lead to vinegar-like off-flavors. Claims that ratios of acetic acid to other fatty acids are important determinants of Cheddar flavor (Ohren and Tuckey, 1969) have not been confirmed (Law, Castanon and Sharpe, 1976).

Generally, free fatty acids have been acknowledged to contribute cheesiness in Cheddar flavor (Forss, 1979). Studies with enzyme-modified cheese suggest that increased free volatile fatty acids in lipase-treated American Cheddar increase its flavor intensity, provided that high rancidity-inducing lipase levels are avoided (Kosikowski and Iwasaki, 1975; Sood and Kosikowski, 1979). Harper, Wang and Kristoffersen (1979) showed that free fatty acids from C₄ to C₁₀ were produced in
vegetable fat modified Cheddar cheese, but their levels were significantly lower than those in the milk fat cheeses. Cheddar cheese manufactured with vegetable or mineral lipids appears not to develop characteristic flavor (Foda et al., 1974; Harper, Wang and Kristoffersen, 1979).

Fat plays a very important role in the development of a good texture, and it is well known that a higher fat content leads to a less firm and elastic body. These differences can be explained by the presence of more protein matrix in the cheese (Emmons et al., 1980).

Native milk lipase, microbial lipases, and lipases originating from somatic cells or blood plasma can hydrolyze triglycerides of milk fat to release FFA (Downey, 1980; Jurczak et al., 1981). Activity of microbial lipases is the predominant source of excessive lipolysis in cheese (Kuzdzal-Savoie, 1980). Psychrotrophic bacteria that possess heat stable lipases are also potential contributors to free fatty acid production during cheese ripening (Deeth and Fitz-Gerald, 1975; Law, Sharp and Chapman, 1976). Fluckiger (1981) reported that both longer milk storage times and increased use of large refrigerated silos that are agitated by air injection promote growth of aerobic psychrotrophic bacteria in raw milk. Cogan (1980) reviewed several studies that discussed the deteriorative
effect of heat-resistant microbial lipases on cheese quality and he found that psychrotrophic bacteria produce most of the heat-resistant microbial lipases.

Although free fatty acids (FFA) have been established as important flavor compounds in several of the aged cheese varieties with more pronounced flavors, only limited numbers of studies dealing with the definitive quantitative aspects of FFA in cheeses have appeared. The absence of an accurate, routine analytical method for quantification of individual major FFA in cheeses has hampered progress of definition of the role of FFA in cheeses (Aston et al., 1982). Some apparently fine-flavored Cheddar cheeses have low concentration of the short-chain FFA (Woo et al., 1982). Free fatty acids derived from milk fat are generally believed to contribute to the flavor of most aged cheese (Badings et al., 1980; Law, 1981; Law, 1982; Adda et al., 1982). Law (1982) concluded that mixtures of alkanoic acids with carbon chains from C₂ to C₈ or C₁₀ can impart cheese-like flavors either to naturally maturing cheese or in flavor mixtures for process cheese, but that their contribution to the aroma and the special character of Cheddar cheese is unproven.

Generally, young Cheddar cheese contains low FFA, and aged, desirably-flavored Cheddar cheese has intermediate concentrations of individual FFA. However,
when conditions permit development of more FFA in Cheddar, rancid off-flavors are readily apparent. This is specifically true for butyric acid (Woo et al., 1984). Hydrolytic rancidity flavor defects in Swiss, Brick, and Cheddar cheeses were signaled by measurement of high concentrations of individual short-chain free fatty acids (Woo et al., 1984). Bynum et al. (1984) reported that large increases of total free fatty acids in a Cheddar cheese sample correlate with development of rancid flavors.

**Importance of Ketones, Lactones and Alcohols in Cheddar Cheese Flavor**

Other fat-derived flavor compounds implicated in Cheddar flavor include ketones and lactones. The odd-numbered methyl ketones do not appear to be vital flavor compounds since they are absent from mature flavored experimental cheeses made with only starter bacteria (Law, Castanon and Sharp, 1976). Manning (1979) indicated that 2-Pentanone concentrations in normal cheese are a good index of cheese age, but the compound is not necessarily involved in flavor.

The methyl ketone, 2-butanone, has been widely reported to be present in Cheddar cheese (Scarpellino and Kosikowski, 1958; Lawrence, 1963; Kroger and Patton, 1964). Day, Bassette and Keeney (1960) estimated the concentration of 2-butanone to be 12.5 ppm in mature
Cheddar cheese, the highest concentration of any methyl ketone present. Bills, Willits and Day (1966), however, found up to 19 ppm of 2-butanone in four of 10 Cheddar cheeses chosen at random. Keen and Walker (1974) also observed wide variations in the concentrations of 2-butanone even in Cheddar cheeses made using the same single-strain starter (*Streptococci cremoris* AM2).

Scarpellino (1961) suggested that 2-butanone contributes to the desirable flavor complex of Cheddar cheese whereas the reduction product, 2-butanol, is associated with off-flavor development. A pathway has been proposed for the formation of 2-butanone from 2,3-butyylene glycol (Scarpellino and Kosikowski, 1962). The compound 2-butanone is never found at concentrations higher than its threshold (Kinsella, 1969) and tends to disappear as cheese ages (Keen et al., 1974). Keen and Walker (1974) suggested that an adventitious non-starter bacterium in the Cheddar cheese may be responsible for its formation.

Although lactones have been shown to improve blue cheese flavor, their contribution to Cheddar flavor is less clear and no definitive evidence exists linking lactone concentrations in maturing cheese with flavor quality or intensity (Law, 1982). However, they are regarded as important by some flavorists, as evidenced by their inclusion in synthetic cheese flavor formulations (Henning, 1970; Smith et al., 1977).
The fruity defect is relatively common in Cheddar cheese and although it can be pleasant at low intensities it usually becomes unpleasantly strong in mature cheese. The defect occurs in cheeses which contain high ethanol levels (147-1527 ppm) (McGugan et al., 1975). Esterase activity in the cheese catalyses the reaction between volatile fatty acids and ethanol to produce esters whose flavor/aroma is reminiscent of pear drops. Ethyl butyrate and ethyl hexanoate (caproate) appear to be the main fruity esters in Cheddar cheese (McGugan et al., 1975; Bills et al., 1965). Manning (1979) reported that the high concentration of ethanol and H₂S found in cheese headspace have been related to the flavor defects "fruity" and "sulfide" respectively. The available microbiological evidence suggests that the fruity defect can be caused by the starter streptococci themselves, by heterofermentative nonstarter lactic acid bacteria, or by enzymes from psychrotrophic bacteria (Law, 1982).

Importance of Organic Acids in Cheddar Cheese Flavor

The aroma and flavor of quality Cheddar cheese is attributed to a delicate balance of organic acids and volatile organic chemicals produced as metabolites by culture bacteria during fermentation (Marsili, 1981).

Organic acids occur in dairy products as a result of the hydrolysis of butterfat (fatty acids), direct addition as acidulants (e.g., citric, lactic, and
propionic acids), normal bovine biochemical metabolic processes (e.g., citric, hipuric, uric, orotic, and ascorbic acids), or bacterial growth (e.g., pyruvic, lactic, acetic and propionic acids). Quantitative determinations of these acids in dairy products is important to flavor studies, for nutritional reasons, and as an indicator of bacterial activity (Marsili, Ostapenko, Simmons and Green, 1981).

Marsili and coworkers (1981) did analyses for the various cultured dairy products and reported that bacterial activity reduced the concentration of hipuric acid, orotic acid, and in some cases, citric acid. Also they monitored the concentration of the following organic acids (ug/g) in sharp Cheddar cheese using HPLC as follows: orotic (4.9 ± .2); citric (25 ± 2); pyruvic (26 ± 2); lactic (5140 ± 40); uric (16.7 ± 1); formic (420 ± 40); acetic (600 ± 10). Several workers have reported orotic acid to be readily utilized by various bacteria used in the fermentation of dairy products (Chen and Larson, 1971; Empie and Melachouris, 1977; Larson and Hegarty, 1979).

Fermentation and flavor development are achieved through the use of a starter culture containing the acid producing *Streptococcus cremoris* which ferment lactose thereby producing lactic, acetic, propionic, formic and pyruvic acids and traces of acetaldehyde, ethanol, and
carbon dioxide (Vedamuthu, 1977). Vasavada (1979) has demonstrated that flavor of cultured dairy products depends not only on the concentration of flavor compounds but also on the ratios in which these compounds are present. Marshall and Harmon (1978) and Zandstra and deVries (1977) utilized colorimetric analysis and suggested measurement of the pyruvate content in milk as an index of bacteriological quality.

Cheddar cheese ripening showed dramatic decreases in lactose during pressing with subsequent increases in lactic and citric acids. Further ripening yielded slight increases in lactic acid, stabilizing of citric acid, and trace development of other acids. Extraction and analysis of other varieties of cheese indicated additional acids including pyruvic, acetic, propionic and butyric acids. Lactic acid was the predominant acid in all cheeses analyzed (Irwin et al., 1984).

Moisture, Protein, Fat and Salt Contents of Cheddar Cheese as Affected by Aging at 7°C

Moisture, protein, fat and salt contents were significantly affected by ripening period. Moisture content decreased while fat and salt contents increased with age of cheese and no trend was observed on protein content (Barraquio et al., 1982). A decrease in moisture content was noted as the cheese ripened and this could be attributed to continuous loss of water through
evaporation and displacement of water as the salt penetrates (Geurts et al., 1974). The more mature cheese had higher salt levels than the younger cheese and this was attributed to loss of moisture and the permeation of salt from the rind to the inner portion of the cheese as it ripens (Gomes, 1977). Barraquio et al. (1980) established a significant increase in fat content as ripening proceeded. The increase in fat content towards the latter part of ripening was attributed by Gomes (1977) to the decreasing moisture content.

**Effect of Protein Degradation on Cheddar Cheese Flavor**

Cheese ripening is a complex process; it includes both physical and chemical reactions. Proteolysis, lipolysis and lactose decomposition are necessary to obtain the desired texture, body and flavor in the final product (Schmidt, 1976). Schormulles (1968) summarized the proteolysis of cheese as protein breakdown, peptide and free amino acid formation, and decomposition of amino acids by decarboxylation transamination, or other metabolic mechanisms during ripening. Proteolytic enzymes from starter bacteria seem to be necessary for the formation of amino acids which serve as a "background" for the normal cheese flavor (Reiter et al., 1969). Kinsella and Hwang (1976) pointed out that proteolysis is important for production of amino acids, which act as precursors of flavor compounds and enhance
methyl ketone production in blue cheese. The extent of protein breakdown has been used as a crude index of cheese ripening (Arnott et al., 1957; Vakaleris et al., 1959; Puham et al., 1960; Morris et al., 1963; Tawah et al., 1966; and Abd El-Salam et al., 1973).

The proteins of fresh curds (mainly caseins) provide a second source of flavor compounds as they are degraded slowly by the proteinases and peptidases of the starter bacteria. Many of the individual free amino acids released by this process have distinctive tastes and their predominance in certain foods is thought to confer characteristic flavor (e.g., methionine in uni, glycine in crab) (Kirimura et al., 1969), but it is generally accepted that their role in Cheddar cheese is one of conferring a savory background flavor as a complex mixture (Fryer, 1969; Leibich et al., 1970).

Peptides are often cited rather loosely as contributing various flavor notes to cheese, but evidence is not definitive and the range of flavors associated with synthetic peptides (sour, bitter, tasteless) is hardly the basis for high expectations of this class of compounds. Nevertheless, the fact remains that it is possible to intensify flavor and/or accelerate flavor development in Cheddar cheese by adding exogenous proteinases to speed up casein breakdown (Sood and Kosikowski, 1979; Law and Wigmore, 1982).
Law (1982) reported that the correlation between increased typical flavor intensity and increased proteolysis only holds good over a limited range and the choice of proteinase type is critical. From his experience that neutral proteinases are ideal and that acid proteinases produce excessive amounts of bitter peptides even at low concentrations in cheese.

There are two possible reasons for the intensification of cheese flavor through progressive proteolytic action. First, the direct result of increasing free amino acid concentrations in cheese is to increase its savory taste and provide substrates for the release of volatile sulphur compounds. The importance of such compounds has long been suspected and their unique position within the wide spectrum of Cheddar flavor volatiles was demonstrated by McGugan and coworkers (1968). They showed that both flavorless cheese produced without starter bacteria and mature normal cheese contained the same neutral volatile compounds in similar quantities, with the notable exception of sulphur compounds.

Later, in a series of papers beginning in 1973, Manning accumulated evidence suggesting that methanethiol (derived from methionine by nonenzymic reactions) was the key compound in Cheddar aroma (Law, 1981). Hydrogen sulphide was also considered important but its
concentration was not critical unless very large amounts were present, when "sulphide" flavor defects became noticeable. Attempts to synthesize Cheddar aroma with these compounds have been unsuccessful probably because their extreme volatility causes their concentrations in synthetic mixtures to change rapidly and become unbalanced; the individual aromas of both compounds are extremely unpleasant. Presumably the matrix of cheese is such that these volatiles are bound or dissolved and they are only released when the cheese is masticated.

McGugan and coworkers (1979) suggested that the binding equilibria of flavor and aroma compounds to cheese proteins was an important factor in determining overall cheese flavor intensity. These investigators suggested that the correlation between flavor intensity and proteolysis may be due to the progressive weakening of flavor binding as cheese proteins are degraded to smaller fragments.

**Compounds Causing the Bitter Taste in Cheddar Cheese**

Bitterness is defined by Crawford (1977) as a condition of flavor which is noticeable to the taste but not by smell. Some 50 years ago, Kelly (1932) reported that bitter flavor in Cheddar cheese was due to the breakdown of protein to peptides and he linked strains of *Strep-tococcus cremoris* starters with the defect. Shortly thereafter, Phillips (1935) reported that bitter flavor
was most frequently found in very acid cheese and in cheese having a pH of \(< 5\) in the first week of curing.

Bitterness, a flavor defect of cheese and of other cultured dairy products, has been found to be due to the accumulation of bitter-tasting peptides (Raadsveld, 1953) which are formed during the normal breakdown of casein by rennet and by bacterial proteolytic enzymes (Stadhouders, 1960; Emmons, 1962a,b; Gordon and Speck, 1965b; Harwalkar and Seitz, 1971; Harwalkar and Elliott, 1971; Richardson and Creamer, 1973; Hamilton et al., 1974). Bitter peptides may be produced from casein by the action of a variety of proteolytic enzymes, by microorganisms and by chemical hydrolysis (Tokita, 1969).

All the bitter compounds which have been extracted from cheese and other cultured dairy products have been identified as peptides originating from \(\alpha\) or \(\beta\)-casein. These peptides are fragments split off the casein molecules by proteolytic enzymes present in the rennet extracts and in the starter bacteria (Jago, 1974). The structures of two bitter peptides, one isolated from Cheddar cheese (Hamilton, Hill and Van Leeuwen, 1974) and another from a milk culture of a starter organism (Gordon and Speck, 1965a; Sullivan, Kieseke and Jago, 1971) are illustrated below:

\[ \begin{align*}
\text{Gln-Asp-Lys-Ile-His-Pro-Phe-Ala-Gln-Thr-Gln-Ser-Leu-Val-Tyr-Pro-Phe-Pro-Gdy-Pro-Ile-Pro} \\
\text{Gln-Gln-Pro-Val-Leu-Gly-Pro-Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val.}
\end{align*} \]
These peptides, which originated from B-casein, have special chemical properties which enable them to react with the taste buds at the back of the tongue to give the sensation of bitterness (Jago, 1974). Other bitter peptides with similar chemical properties have also been isolated from Cheddar cheese and found from αs or β-casein (Hodges, Kent and Richardson, 1972). Bitter peptides are released from the casein molecules primarily by the action of the rennet and bacterial proteinases. However, the bacterial peptidases may also contribute to the formation of bitter peptides by reducing the size of peptides initially too large to give a bitter taste (Sullivan and Jago, 1972; Sullivan, Mou, Rood and Jago, 1973). Bitterness, a common flavor defect of Cheddar and Gouda cheese, results from the accumulation of bitter-tasting peptides formed by the action of proteolytic enzymes on casein (Fryer, 1969; Stadhouders and Hup, 1974; Lawrence, 1976).

Bitter and astringent fractions have been isolated from cheese by Harwalkar and Elliott (1971). Bitterness and fruity off-flavors in Cheddar cheese, caused by the production of bitter peptides and ethyl esters of fatty acids respectively by starter bacteria were discussed by Sandine and his coworkers (1972).

Proteolysis in cheese has an important negative influence on flavor quality, related to the caseinolytic
action of the starter streptococci and the residual rennet. The bitter defect in cheese is caused by an accumulation of peptides containing a high proportion of hydrophobic side chains (Harwalkar, 1972; Matoba et al., 1972; Richardson, 1973). Opinions differ as to the importance of proteolysis by mesophilic starters in the production of bitter defects in cheese. Early hypotheses suggested that bitter peptides were produced by chymosin and that the so-called "bitter" starters were those which had insufficient peptidase activity to break down the bitter peptides to nonbitter peptides and amino acids (Czulak, 1959).

The situation is more complex. While it is true that chymosin produces bitter peptides from casein, the starter proteinases can also do this and, indeed, can produce small bitter peptides from nonbitter, casein derived peptides (Figure 1) (Lowrie et al., 1972; Lowrie et al., 1974). It is suggested that this latter process was the single most important determinant in bitterness development, and that "fast" starters which multiplied at relatively high cooking temperatures during Cheddar manufacture were the most likely to give bitter cheese, simply because the resultant high cell numbers contributed large quantities of bitter peptide-producing proteinases.

Lowrie and coworkers (1974) supported this hypothesis with experimental evidence that bitter
starters could be made to produce non-bitter cheese if their number in curds were restricted by controlled bacteriophage infections or by higher cooking temperatures. Conversely the slow nonbitter starters made bitter cheese if they were allowed to multiply to high cell numbers by altering the manufacturing process. Direct evidence for the involvement of starter cell wall proteinases in bitterness development was provided recently by the observation that proteinase-deficient variants of "fast" starters produce less bitterness in cheese than their parent strains even when total starter cell populations are high (Mills et al., 1980).

The factors controlling bitter defects in Gouda cheese appear to be more complex, since the starters generally reach high populations in curds at the relatively low cooking temperatures used for this variety. Stadhouders and Hup (1975) showed that factors influencing the retention of chymosin in Gouda curd (e.g., cooking temperature, initial milk pH) also influenced the tendency of the cheese to become bitter. They emphasized that some starter strains produce more bitter peptide-degrading peptidases than others. It is not known whether these are specific peptidases confined to nonbitter strains or general peptidases present at different levels. Chiba and Sato (1980) identified both dipeptidase and amino-peptidase activity in debittering
Figure 1. Model for bitterness development in Cheddar cheese (Lowrie and Lawrence, 1972). Bold arrows indicate the important stages (1 and 2). Broken lines indicate stages of lesser importance.

fractions of cell-free extracts from starter streptococci but individual enzymes were not isolated. It appears, then, that proteolysis by mesophilic starters is important in producing the bitter defect in cheese but its contribution is different depending on the cheese variety in question.

Factors Involved in Bitterness

The excessive bitterness in some cheese is objectionable, and, therefore, of considerable economic
significance. Excessive bitterness is influenced by the cheese making procedure and by factors such as strain of starter culture, rennet concentration, heat treatment of the milk, acidity or pH of cheese and salt concentration (Emmons et al., 1962a,b).

Lawrence and Gilles (1969) also attributed an important rule to the starter cultures used and concluded that a number of factors were involved. These were the proteinase activities of both starter and rennet enzymes and the effects of rate of acid production, pH, salt and moisture on these activities.

Influence of Starter on Bitter Flavor Production

The early hypothesis by Czulak (1959) stated that bitter flavor develops in Cheddar cheese when the starter bacteria lack the peptidases necessary to degrade the peptides produced by rennin; the peptides accumulate and yield a bitter flavor.

Czulak (1959) and Fryer (1969) postulated that the accumulation of bitter-tasting polypeptides might be due either to increased proteolytic activity of the rennet enzymes at lower pH level (<5) and the slower hydrolysis of the polypeptides by bacterial proteinases to the amino acid stage at the lower pH levels or to incomplete degradation of the peptides.

Work by Emmons et al. (1962a,b) showed that some starter strains that produced bitterness were not
affected significantly by the pH; whereas, others were
greatly affected. Evidence was presented that bitterness
was caused by peptides unhydrolysed to amino acids due to
a deficiency of peptidases. This view was also supported
by the later findings of Czulak and Shimmin (1961) and
Stadhouders (1962).

Further work by Emmons et al. (1962a,b) to test the
effect of combining starter strains showed that combina-
tions of bitter and non-bitter strains of Streptococcus
cremoris sometimes yielded non-bitter cheeses, and
intensity of bitterness usually decreased as the
proportion of the non-bitter cheese-producing strain in
the starter increased. They also noted that the
intensity of bitterness was higher in cheeses where the
average chain lengths of trichloroacetic acid (TCA)-
soluble peptides and amino acids were greater. Jago
(1962) showed that differences between "bitter" and "non-
bitter" strains was not due to their different
proteolytic activities towards milk proteins, but to the
inability of "bitter" strains to hydrolyse the bitter
peptides produced by rennet. Subsequently, Gordon and
Speck (1965a,b) found that in milk, "bitter" strains of
Streptococcus cremoris possessed greater proteolytic
activity than "non-bitter" strains.

Yamamoto and Yoshitake (1962) demonstrated the
proteolytic function of starter organisms in cheese-
ripening by comparing the number and amount of free amino acids produced in cheeses made with and without starter. They found that cheeses made with starters had higher amino acid content than non-starter cheeses.

Gordon and Speck (1965a) studied the ability of *Streptococcus cremoris* to produce bitterness in milk. Strain HP developed bitterness from the casein component of milk. This strain also produced more TCA-soluble nitrogen at 22 and 32°C than non-bitter strains, and furthermore, the bitter strain showed continued proteolytic activity after the viable population declined. Thus, proteolytic enzymes released by cells were capable of continued action on milk casein; rennet was not essential for production of bitterness in milk. Harwalkar and Seitz extracted bitter flavor components from skim milk cultures of *Streptococcus cremoris*; casein was essential for production of the bitterness.

Lawrence and Gilles (1969) reported that "slow" starter never gave bitter cheese even when they were necessary to produce a high rate of acid production or where the salt-in-moisture level and pH would almost certainly result in bitterness when a "fast" starter was used. Also reported earlier that certain strains of both *Streptococcus cremoris* and *Streptococcus lactis* may produce bitterness in cheese. This contradicted earlier reports that only *Streptococcus cremoris* was involved in bitterness.
Lawrence and Pearce (1968) reported that certain single-strain starters, e.g., coded AM₁ and AM₂ which had slower than normal acid production rates always gave good-flavored Cheddar cheese free from bitterness under normal commercial conditions.

Sullivan and Jago (1970a) hypothesized that bitterness resulted from the formation of pyrrolidonecarboxylic acid (PCA, which is a cyclic form of glutamic acid) at the N-terminal end of a hydrophobic peptide derived from casein during proteolysis. Also, they suggested that non-bitter cultures may possess the enzyme pyrrolidonecarboxyl peptidase (PCP) to hydrolyze the peptide bond joining PCA residues to the remainder of the peptides and proteins. In support of this idea, these authors subsequently showed (1970b) that Streptococcus cremoris MLI, a strain which produces non-bitter cheese, contained PCP activity; cell free extracts liberated free PCA when incubated in the presence of two different synthetic PCA containing peptides.

The New Zealand workers (Lowrie and Lawrence, 1972; Lawrence et al., 1978) suggested that the starter streptococci are predominantly responsible for development of bitterness in cheese. The mechanism consisted of three major steps depicted in their model.

1. Degradation of casein by rennet to produce a pool of high molecular weight peptides which are mostly non-bitter.
2. Some of these peptides are hydrolyzed by the proteases of starter streptococci to low molecular weight peptides which are bitter.

3. The third stage concerns further degradation to non-bitter peptides and amino acids by peptidases of starter streptococci.

The essential feature of this hypothesis is that all starter streptococci are potentially bitter and non-bitter. The presence or absence of bitterness depends on the influence of manufacturing conditions on particular starter strains.

The use of cultures containing different proportions of proteinase positive (prt⁺) and proteinase negative (prt⁻) variants in Cheddar cheese making has allowed the level of starter proteinase to be varied while keeping the total concentration of starter cells in the curd at salting constant. Cheeses with 45-75% prt⁻ cells developed significantly less bitterness than cheeses containing only prt⁺ cells, thus providing direct evidence that the level of starter proteinase has a role in bitterness development in Cheddar cheese (Mills and Thomas, 1980).

In cheese, these bitter peptides are apparently degraded to non-bitter products by the starter streptococci during the ripening period. However, some starter strains (described as "bitter") are unable to remove the bitter which thus persist, giving the cheese a
bitter taste (Emmons, McGugan, Elliott and Morse, 1962a; Emmons, McGugan, Elliott and Morse, 1962b; Lawrence and Pearce, 1968; Lawrence and Gilles, 1969).

2.) Influence of Rennet on Bitterness

In referring to the use of microbial rennets in cheese-making, Kikuchi and Toyoda (1970) mentioned the bitter taste frequently found in cheese made with microbial rennets produced by *Mucor pusillus lindt* and *Bacillus polymyxa* and suggested that the formation of bitterness was an inherent characteristic of the enzymes and that more satisfactory cheese quality might be obtained by modifying the manufacturing methods to suit the microbial coagulants.

Lawrence and Gilles (1971) referred to trials in New Zealand which showed that bitterness scores in Cheddar cheese made with "fast" single-strain starters HP, ML8, Z8, BA₁, E8 and ML₁ were directly proportional to the amount of rennet added. The same workers reported that 2 to 3-fold increase in the amount of rennet caused no increase in bitterness when "slow" starters AM₁, AM₂ and US₃ were used.

Lawrence and Gilles (1971) also found that the bitterness of cheese manufactured with the bitter strains was directly proportional to the amount of rennet used. This was in contrast to cheese manufactured with the non-bitter strains as a 2 or 3 fold increase in the
amount of rennet used. This did not produce a bitter cheese. Lawrence and Gilles (1971) put forward the practical suggestion that the amount of rennet used in Cheddar cheese making could be lowered by about 20% so reducing the tendency to bitterness by raising the temperature of milk at rennet addition by 1-2°C.

Ohmiya and Sato (1972) concluded that rennin contributed less to hydrolysis than starter enzymes.

Lawrence, Creamer, Gilles and Martley (1972) confirmed that the degree of bitter flavor produced in Cheddar cheese made with "fast" acid producing starters and calf veil rennet was approximately related to the amount of rennet used. It is interesting to note that the same workers found that "fast" starters produced less bitterness with coagulant derived from Mucor michei (microbial rennet which is called Rennilase) than calf rennet. However, all microbial rennets were not alike in this respect as another microbial rennet was found to produce bitter cheese even with non-bitter starter (Jago, 1974).

3.) Influence of Cheesemaking Conditions
   A.) Pasteurization Temperature of Milk

Lawrence and Gilles (1969) identified in their analysis on cheese made at the NZ DRI over the period 1957-69, that the use of more vigorous heat treatment conditions (i.e., 72.2°C for 15 sec) introduced in 1961/2
in place of a flash heat treatment led to an increased incidence of bitterness with starter HP. In explaining this, they suggested that the lower heat treatment conditions in use previously had allowed a significant number of bacteria to survive in the milk and develop end products which masked the bitterness.

Dulley (1974) found that when milk was pasteurized at 73°C for 15 sec, approximately twice the rennet was retained in the resulting cheese compared with raw milk cheese or that made from milk pasteurized at 65°C for 15 sec. The presence of higher levels of rennet might cause a higher level of bitterness in the cheese made from the milk heat treated at the higher temperature.

Stadhouders and Hup (1975) reported that cheese made from more intensively pasteurized milk (82 vs. 74°C for 10 sec) had a higher rennet content and a higher score for bitterness than cheese made from milk subjected to a lower heat treatment. Also, they reported that rennet content and bitterness were both increased by (i) lower pH in the cheese milk, due to poor quality or to ripening, (ii) lower cooking temperature in the range 30-38°C, or (iii) lower salt content. Stadhouders et al. (1977) have offered a much needed accurate method to measure rennet content.
B.) Cooking

Lowrie, Lawrence, Pearce and Richards (1972) indicated the scald temperature used in cheese-making affected the level of bitterness in cheese. The higher cooking temperatures of 39.4°C gave cheese which was less bitter than the control (37.8°C).

It is clear that starter bacteria have a direct role in bitterness development in Cheddar cheese since use of higher cooking temperatures leading to relatively low cell densities reduced bitterness development (Lowrie et al., 1972). A lower cell density in the cheese will result in reduced levels of both cell wall-associated starter proteinase (Thomas et al., 1974; Exterkate, 1975) and intercellular proteinases and peptidases (Exterkate, 1975). It is presumed that starter bacteria lyse during cheese ripening and the intracellular enzymes are released into the curd matrix.

As higher cooking temperatures are used, so the frequency and intensity of bitter flavor developed in the cheese is lowered. This holds true when cooking temperatures vary from 35-39°C (e.g., Cheddar cheese). The phenomenon is explained by the reduced amount of rennet retained in cheese when a higher cooking temperature is applied. Above cooking temperatures of 35°C (35-39°C) in addition to the reduced rennet content, the decreased maximum number of streptococci and the decreased content
of their proteases producing bitter peptides (if present) may also be considered to be the cause of the reduced bitter flavor (Exterkate, 1976a,b).

C.) **pH, Salt, Moisture**

Bitterness has been observed most frequently in cheese of low pH (Czulak, 1959; Emmons et al., 1962b; Lawrence and Gilles, 1969).

One of the most important findings by Lawrence and Gilles (1969) was that in the case of cheese made with HP during the period 1964-68, the average salt-in-moisture levels of the non-bitter cheese were considerably higher than those in bitter cheese of the same acidity above and below pH level 4.95.

Lawrence and Gilles (1969) studying bitterness in New Zealand Cheddar cheese over 10 years noted that the defect was dependent on two main factors, the rate of acid production and the "salt-in-moisture" level. They noted that fast acid production of *Streptococcus lactis* and *Streptococcus cremoris* strains had a tendency to yield bitter cheese. Also, whether or not bitterness was produced by a fast starter was determined by the salt-in-moisture level and to a lesser extent by the pH of the cheese at 14 d. They suggested that starter proteinase and rennet activity were related to the solubility of paracasein at a specific salt concentration and pH. In follow-up studies, Lawrence et al. (1970) studied the
influence of type of starter and manufacturing conditions on bitterness in Cheddar cheese starters which yielded the best flavored cheese. The best starters were slower acid-producing types requiring about 6.5 h. from set to salt, while faster strains (4.5 to 5 h. from set to salt) had a tendency to produce bitter cheese. By combining slow and fast strains (2:1, respectively) the incidence of bitterness was acceptably reduced.

A report by Fox and Walley (1971) helps to explain the role of salt in preventing bitterness in Cheddar cheese. Sullivan et al. (1971) recently demonstrated an effect of pH on the ability of "bitter" and "non-bitter" strains to remove bitterness from bitter peptides extracted from tryptic hydrolysates of casein. They reported that strain HP (Bitter) of Streptococcus cremoris was unable to reduce the level of bitterness in the bitter peptide extract at pH values below 5.5, while strain MLI (Non-bitter) could do so at all pH values above 4.5. This report provides evidence that pH is a determining factor in the degradation of bitter peptides by individual strains of starter streptococci.

When the initial pH of the cheese milk is decreased, more rennet remains in the cheese and there is more chance for bitter flavor to develop. A lower initial pH may occur in practice when the milk is of inferior quality and acid formation has taken place, when the milk
is preripened with starter or when a larger amount of
starter has been used. Another explanation may be that
the low pH has changed the calcium phosphate/casein
complex so that bitter peptides are more easily produced
from it. Salt reduces the intensity of bitter flavor
development in Gouda cheese and a higher ripening
temperature was found to promote it (Exterkate, 1976a,b).

D.) Bacteriophage

An interesting comment was made by Lawrence and
Gilles (1973) on the role of bacteriophage in determining
cheddar cheese flavor. This work showed that when
"bitter" starters (e.g., N. Z. single-strain starters HP
and Zg) were used on two or more consecutive days, almost
without exception cheese made on the first day was more
bitter and less acceptable in flavor at six months of age
than cheese made on the following day. This suggested
that bacteriophage was responsible for the longer times
of manufacture on the subsequent days and that by
reducing starter numbers by a high level of bacteriophage
prevented or reduced the chance of cheese being bitter
and unacceptable.

The New Zealand workers, Lowrie, Lawrence, Pearce
and Richards (1972) and later Lowrie, Lawrence and
Peberdy (1974) confirmed the importance of bacteriophage
infection in restricting the development of bitterness by
starters. Bacteriophage contamination during cheese
making at levels which restricted starter growth in the final stages of manufacture without markedly affecting acid production had a striking effect on cheese flavor, especially intensity of bitterness (Lowrie, 1976).

**Control of Bitterness Levels in Cheese**

Jago (1974) concluded from evidence obtained by a number of workers that bitterness in cheese was due to the formation of bitter peptides cleaved from casein through the action of proteolytic enzymes of starter bacteria and rennet. In his view, the control of bitterness involves methods to reduce the proteolytic activity in the cheese so that the bitter peptides produced do not exceed the threshold level necessary to result in a bitter taste. Breakdown of bitter peptides to non-bitter products is also a factor in determining the concentration of bitter peptides in cheese but so far, there did not appear to be any method which could be easily applied to increase the degradation of bitter peptides in cheese.

To reduce proteolytic activity in cheese so that bitterness is not formed, Jago (1974) suggested several measures:

(a) Limit the starter cell population in the curd by using starter strains whose growth was restricted by the scald temperatures used in the process.

(b) Reduce the amount of rennet required by increasing the coagulation temperature 1-2°C.
(c) Produce a high pH and a high salt-in-moisture level in the cheese.

To obtain the required acid production rate by heat-sensitive (non-bitter) starters, Jago (1974) suggested either using larger amounts of inoculum or the combination in the vat of heat-resistant (bitter) strains and non-bitter strains in the ratio of one part bitter to two parts non-bitter.

Lawrence and Gilles (1969) have shown that a high salt-in-moisture reduces the incidence of bitterness in cheese manufactured with a bitter starter. This appears to be due to the fact that sodium chloride inhibits the degradation of B-casein (a major source of bitter peptides) by the rennet and bacterial proteinases (Fox and Walley, 1971; Sullivan and Jago, 1972).

Bitter taste in cheese is prevented by adding to the inoculum strains of lactic acid bacteria capable of breaking down bitter products formed during splitting of casein (Zvyagintsev et al., 1972).

**Application of Stepwise Analysis for Judging Ripening of Cheddar Cheese**

Water extracts from 41 Cheddar cheese samples of different ages were analyzed by high pressure liquid chromatography on the C8 Adsorbosphere reverse-phase column eluted with .1 M phosphate buffer, pH 6.0. Stepwise discriminant analysis classified cheeses into mild, medium, old, and extra-old. Discriminant functions
were calculated for classifying unknown samples from their high pressure liquid chromatography data. Majority of the peaks separated by high pressure liquid chromatography consisted of protein degradation products (Anne-Marie and Shuryo, 1984).

Cheese flavor is derived from a complex balance of several compounds (Aston et al., 1982). The importance of the volatile compounds in cheese aroma has been reported (Kristoffersen, 1973; Manning et al., 1973; Manning et al., 1977). Multiple regression analysis was used to correlate individual flavor components to the flavor of Cheddar cheese (Manning, 1979). The contribution of methyl ketones and lactones to Cheddar flavor was investigated by Walker and Keen (1974) and Wong et al. (1975), respectively. Importance of nonvolatile fractions has not been recognized until recently. McGugan et al. (1979) extracted the residue after removing fat from Cheddar cheese by centrifugation with a combination of methanol methylene chloride, and water. The separated methanol-water layer containing water-soluble compounds and fat separated by centrifugation and deodorized by steam distillation were compared for flavor. Cheddar cheese flavor was characteristic in the water extract. No difference between deodorized and undeodorized fat fractions was significant, indicating that the loss of volatiles was not important for cheese flavor intensity.
Pattern recognition techniques for multivariate analysis have been used in chemistry, microbiology, and medicine for identification of chemical compounds, classification of bacteria species, and diagnoses of diseases, respectively. By applying principal component analysis, linear discriminant analysis, and multiple regression analysis to gas chromatography data, Aishima (1979) reported that eight brands of soy sauce samples were classified correctly into eight groups or three groups of good, regular, and inferior quality. Unknown samples also were classified correctly.
CHAPTER III
MATERIALS AND METHODS

Part I

The first phase of this research concentrated on the effects of psychrotrophs in raw milk on Cheddar cheese quality.

Cheddar Cheese Preparation

The procedure of manufacture was a slight modification of Kosikowski (1978). The only requirement stipulated by the design of the experiment was a milling acidity of .53% lactic acid and a moisture content of cheese of 36-38% which would lend commercial validity to the study.

1) Grade A raw milk of high quality was inoculated with one of two levels (10,000 and 100,000/ml) of one of two psychrotrophs (Pseudomonas fluorescens 27; Pseudomonas fluorescens 103) and incubated at 7°C for 48 h. The contaminated milks (and a control milk) were pasteurized at 71.7°C for 15 sec (HTST). A total of 110 kg milk was transferred into 378.4 L stainless steel vats.

2) Two 378.4 l cheese vats and all cheesemaking equipment were cleaned thoroughly and chlorinated
immediately prior to manufacture.

3) The milk was added to the vats and heated (steam jacket) to 31.1°C (88°F). The acidity and bacteriological counts (SPC, CVT, and coliform) were determined on each vat of milk.

4) A mixed strain lactic culture (Chr. Hansen's DVS Nos. 1060, 991, 1011 and 980) with a titratable acidity of at least .70% was added.

5) The acidity was checked after 30 min, and, if there were an increase of at least .1%, a double strength Annatto cheese color was added at the rate of 15 ml/454 kg milk. A single strength rennet extract was added at the rate of 120 ml/454 kg milk. This rennet was diluted 1:40 with water prior to addition. The vat was covered to keep the surface warm and clean while the curd was sitting before cutting.

6) After 30-45 min, depending on firmness of curd, the curd was cut, allowed to settle for 15 min, and cooking started.

7) The temperature was raised to 39°C in 30 min (an increase of about 1°C every 4 min). The cheese was cooked at this temperature for one h.

8) The whey was drained when the acidity reached at least .14%.

9) Curd was then cheddared for approximately 2.25-2.5 h.

10) Cheddared curd was milled at acidity of .53% lactic acid, then salted (1.135 kg/454 kg milk).
After pressing overnight (18 h), curd was placed on clean wire head cutter to cut it into .454 kg (1 lb) cheese blocks. Flexible, low temperature red wax that can be peeled neatly off the cheese was used to wax the cheese. Cheese was dipped twice into this wax at about 150-170°F (65.6-76.7°C) for 10 sec. After the wax had firmed, it was properly stamped with the necessary identification. The waxed cheeses were fitted in the appropriate size heat-shrink cryovac bags, which were represented by a transparent rubberized wrapper. The film wrapping shrank significantly upon exposure to high heat. The resulting close adherence to the cheese improved its ability to prevent mold growth and giving a desirable tightness. The package was subjected to relatively low vacuum, 38.1 cm after heat shrinkage at 77°C. The cheese blocks were placed on wooden shelves with clean top and bottom, 85% relative humidity curing room at 7°C (45°F) and "cured" or ripened for 180 d.

Treatment Sampling

On days of analysis, which were d 0, 5, 30, 60 and 180, four blocks of cheese were collected from each treatment and taken immediately to the lab for analysis. One block was analyzed for microbial parameters, followed by the analysis of the other three blocks for chemical components and flavor. A schematic of treatment
preparation is in Figure 2 and testing is outlined in Table 1.
Raw milk

Added psychrotrophs

A. *Pseudomonas fluorescens* 27
   1. 10,000/ml
   2. 100,000/ml

B. *Pseudomonas fluorescens* 103
   1. 10,000/ml
   2. 100,000/ml

C. Control - No added psychrotrophs

Incubate at 7°C for 48 h

Pasteurize at 71.7°C for 15 sec

Cheese manufacture

Microbiological, chemical and flavor determinations on d 0, 5, 30, 60 and 180.

Figure 2. Schematic diagram of treatment preparation and testing for Part I.
Table 1. Outline of Study for Part I

<table>
<thead>
<tr>
<th>A. Microbial tests</th>
<th>G. GCHS</th>
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<tbody>
<tr>
<td>1. SPC</td>
<td>1. Acetone</td>
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<tr>
<td>2. Streptococci</td>
<td>2. 2-Butanone</td>
</tr>
<tr>
<td>3. MRS</td>
<td>3. Ethanol</td>
</tr>
<tr>
<td>4. Coliform</td>
<td>4. 2-Pentanone</td>
</tr>
<tr>
<td>5. CVT</td>
<td>5. 2-Butanol</td>
</tr>
<tr>
<td>B. Physical tests</td>
<td>6. N-Propanol</td>
</tr>
<tr>
<td>1. pH</td>
<td></td>
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<tr>
<td>2. Moisture</td>
<td>H. GC</td>
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<tr>
<td>3. Salt</td>
<td>1. Butyric</td>
</tr>
<tr>
<td>C. HPLC</td>
<td>2. Caproic</td>
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<tr>
<td>1. Orotic</td>
<td>3. Caprylic</td>
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<tr>
<td>2. Citric</td>
<td>4. Capric</td>
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<tr>
<td>3. Pyruvic</td>
<td>5. Lauric</td>
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<tr>
<td>4. Lactic</td>
<td>6. Myristic</td>
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<tr>
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<td>8. Stearic</td>
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<tr>
<td>7. Propionic</td>
<td>9. Oleic</td>
</tr>
<tr>
<td>D. Cheese yield</td>
<td>10. Linoleic</td>
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<tr>
<td>E. Proteolytic activity</td>
<td>11. Linolenic</td>
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<tr>
<td>(Hull test)</td>
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<tr>
<td>F. Sensory evaluation</td>
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</tr>
<tr>
<td>1. Flavor</td>
<td></td>
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<tr>
<td>2. Body and texture</td>
<td></td>
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</table>
Microbiological Enumeration

In order to monitor growth and decline of different microbial populations in Cheddar cheese, the following media were utilized: Tryptone Glucose Extract (TGE) agar for total mesophilic aerobic bacteria (American Public Health Association, 1978); Crystal Violet Tetrazolium (CVT) for gram negative psychrotrophs (Speck, 1976). This test was used rather than the 10 d standard psychrotrophic bacteria count; Violet Red Bile (VRB) agar for total coliform (APHA, 1978); MRS agar for lactobacilli and KF agar for streptococci (Speck, 1976). Enumerations were performed in duplicate with three consecutive serial dilutions using 2% sodium citrate as a dilution blank.

Selection of Pseudomonas fluorescens P27 and P103

Prior to this study, several strains of Pseudomonas were identified using the Oxi/Ferm Tube Method (Roche Laboratories). These organisms were checked for proteolytic activity as described by the American Public Health Association (1978) and lipolytic activity described by Speck (1976). As a result, P. fluorescens P27 and P103 were chosen based on their frequency of occurrence in dairy products and their higher proteolytic and lipolytic activity compared to other strains tested. Prior to inoculating the Oxi/Ferm tube, the oxidase test was performed to separate the oxidative gram-negative rods from the enterobacteriaceae. This is
Figure 3. Schematic diagram of identification of *Pseudomonas fluorescens* and *Enterobacter aerogenes*.
illustrated in Figure 3. The Oxi/Ferm tube permits the inoculation of all media and subsequent performance of nine standard biochemical tests. The numbers corresponding to the positive reactions were totaled and the composite number was then located in the coding manual to identify the organism. Three organisms were listed under one identification value; confirmatory tests were recommended for further separation of the organisms as shown in Figure 4.

Detection of Phospholipase Producing Bacteria by Lecithin Agar

Lecithin Agar (LA) suitable for demonstration of phospholipase activity (McMurray et al., 1972 and Chrisope, Fox and Marshall, 1976) was formulated as follows:

Fraction A contained

3.0 g of Crude Soy Lecithin (Eastman Kodak Co.)
45.0 ml of Distilled Water

Fraction B contained

1.0 g of Tryptone
0.5 g of Yeast Extract
0.5 g of Glucose
1.8 g of Agar
50.0 ml of Distilled Water

Fraction C contained

5.0 ml of 0.1 M CaCl₂

Fraction A was brought to boiling and swirled to form a viscous solution, which was sonicated until homogeneous. Blending of unheated Fraction A in a Waring
OXI/FERM TUBE™

2171

PSEUDOMONAS AERUGINOSA
PSEUDOMONAS PUTIDA
PSEUDOMONAS FLUORESCENS

CONFIRMATORY TESTS

<table>
<thead>
<tr>
<th></th>
<th>PYO</th>
<th>42</th>
<th>GEL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 4. Oxi/ferm tube for identification of *Pseudomonas fluorescens*
blender for 2 min at high speed was also satisfactory. The blended sample was used. The fractions were autoclaved separately for 15 min at 121°C. After equilibration of temperature to 42 to 44°C, the fractions were combined with gentle swirling, A + (B + C). To prevent separation, plates were poured immediately with 12 to 15 ml of the mixture.

Pseudomonas were streaked onto surfaces of lecithin agar plates. After incubation for up to 9 d at 21°C, plates were observed for changes in appearance of the medium.

A culture of Pseudomonas fluorescens producing an opaque zone, suggests phospholipase C activity (formation of water-insoluble diglyceride) while strains of Pseudomonas fluorescens producing a clear zone is attributed to activity of phospholipase A₁ or A₂ (formation of water-soluble lysolecithin).

Psychrotrophic Inoculation

In this study, a method was developed making possible the inoculation of psychrotrophic bacteria in known quantities to raw milk. Several methods and procedures were tested resulting in the following procedure:

1. Seven Trypticase Soy Agar (TSA) slants were prepared in test tubes of equal inside diameter
and slanted to the same angle providing equivalent surface areas.

2. A standard loop (3 mm I.D.) of a stock culture of \textit{P. fluorescens} P27 or P103 was aseptically transferred and spread over the entire surface of each slant (one loop per slant).

3. After incubation at $21^\circ\text{C}$ for 24 h, each slant was washed with 1 ml buffered phosphate solution. This removed growth from the surface of the slant, resulting in a turbid suspension at the base.

4. A 1 ml portion from each slant in step 3 was transferred to corresponding bottles containing 100 ml sterile milk. Milk was previously heat-treated in flowing steam for 1 h and cooled to $21^\circ\text{C}$.

5. These samples were incubated at $21^\circ\text{C}$ for 24 h, followed by plating on TSA.

6. Plates were incubated for 3 d at $21^\circ\text{C}$ to obtain an average count.

7. After establishing the average count in step 6, steps 1 through 5 were conducted with two slants prior to inoculation of raw milk.

8. Duplicate bottles of sterile milk containing the psychrotroph in step 5 were combined in a sterile Whirl-Pack bag (Nasco) immediately prior to inoculation.
9. Inoculation levels were calculated based on the average count in step 6.

10. Stock cultures of *P. fluorescens* P27 or P103 were transferred every 3 mo on TSA slants to maintain cultural activity. In addition, culture purity was verified routinely at time of transfer. TSA plates were streaked and incubated at 21°C for 3 d. Isolated colonies were selected and identified by the Oxi/Ferm Tube method.

The above procedure proved very effective in obtaining desired levels of *P. fluorescens* P27 or P103 in treatment preparations.

**Volatile Organic Analysis**

Volatile organics such as acetone, 2-butanone, ethanol, 2-pentanone and propanol were monitored by gas chromatography with headspace sampling (GCHS) as described by Marsili (1981). Slight modifications were made to maximize separation and accuracy without large increases in program time. The following were equipment and operating conditions utilized in this study: Perkin-Elmer model HS6 headspace analyzer; Perkin-Elmer Sigma 3b gas chromatography with flame ionization detector; Perkin-Elmer Sigma 15 computer console and integrator; samples thermostated at 80°C for a minimum of 15 min prior to vial pressurization for 4 min; injector and
detector temperatures of 140°C and 145°C, respectively; injection time 5 sec; stainless steel column (4 m x 3.2 mm o.d.) packed with 4% Carbowax 20M on Chromosorb G AW DMCS, 80/100 mesh; isothermal analysis at 70°C; and nitrogen carrier gas at a flow rate at 28 ml/min.

Sample Preparation of Volatile Organics

Samples for GCHS were prepared in duplicate as follows. A 2.0 ± .005 gram sample was weighed into a 10 ml vial and sealed by a hand crimper with special closures (Perkin-Elmer Corporation) comprised of butyl rubber PTSE coated septum, star spring and aluminum cap. After sealing, samples were frozen and analyzed at a later date.

Standard Preparation and Quantitation of Volatile Organics

Standards were prepared under refrigerated (3-4°C) conditions to prevent losses of volatile compounds at room temperature, thereby insuring precision. Five aqueous volatile organics standards covering a broad concentration range were prepared from stock solutions. Calibration curves were established to check for linearity and quantitations were based on peak area using the external standard technique described by Marsili (1981a).

Principles of Headspace Sampling

The principles of the system are illustrated in
Figure 5. The three basic steps of headspace sampling: a) equilibration, b) pressurization, and c) transfer of an aliquot of the headspace gas into the column. $P_i$ = carrier gas inlet pressure; $P_v$ = pressure in the vial.

Figure 6. Operational diagram of the Model HS 6 with a SIGMA Series Gas Chromatograph
Figures 5 and 6. After equilibrium is established in the vial (i.e., thermostatting it at 80°C for a minimum of 15 min (step A), a needle connected to the carrier gas inlet is introduced through a rubber cap into the headspace of the vial (step B). If the pressure of the vial ($P_V$) is smaller than the carrier gas inlet pressure ($P_i$), part of the carrier gas flow will enter the vial and pressurize it until it reaches the inlet pressure. This pressurization period (4 min) is precisely controlled.

Next, the carrier gas supply is temporarily cut off for a preset time (step C). This causes the gas to flow from the vial into the column, injecting an aliquot of the headspace. This transfer usually takes a few seconds, after which the needle is removed and the carrier gas flow resumed. Through close control of the temperature, pressure, and time, a highly reproducible sample volume is introduced into the column.

**Organic Acid Analysis**

Organic acids (orotic, citric, pyruvic, lactic, formic, acetic and propionic) were determined by high performance liquid chromatography (Marsili et al., 1981). Due to high levels of citric and lactic acids in Cheddar cheese of this study, an attenuation of 526 was used to calculate their areas precisely while an attenuation of 32 was used to calculate the areas of other organic acids. The following were equipment and operating
conditions utilized in this study as illustrated in Figure 7: Varian Model 5000 liquid chromatograph; 50 ul loop injector; Varian U.V. detector set at 220 nm and detector slit width of 8 nm; Varian 8000 Automatic sampler; Cation exchange column (300 x 78.8 i.d.) packed with Aminex HPX-87 from Bio-Rad Laboratories (Richmond, California); mobile phase of .009 N H₂SO₄ prepared by diluting reagent grade sulfuric acid with distilled H₂O; isocratic analysis at column temperature of 65°C and flow rate of .7 ml/min; and attenuations of 32 and 526.

Sample Preparation of Organic Acids

Samples for organic acid analysis were prepared as described by Marsili et al. (1981) as follows:

1. A 20.0 ± .005 gram sample of grated cheese was weighed and placed in semi micro stainless steel blender jar.

2. 30 ml of .15 N Ba(OH)₂ and .15 N BaCl₂ mixture (100:100 ratio) were added to the blender.

3. 30 ml of .30 N ZnSO₄ were added to the blender.

4. All the mixture was blended for one minute.

5. The blended mixture was divided into two equal volumes in two 50 ml plastic centrifuge tubes and centrifuged at 7000 G for 5 min.

6. Fat was scraped off the top and the clear liquid was poured into another clean 50 ml centrifuge tube.
Figure 7. Operational diagram of the Varian Model 5000 high performance liquid chromatograph with Varian 8000 Automatic sampler.
7. Four drops of $\text{H}_2\text{SO}_4$ were added to each tube to precipitate the barium and prolong the column life.

8. Both tubes were filtered using a Whatman 40.

9. The filtrate was collected into a 100 ml volumetric flask and the volume was brought to 100 ml using distilled water.

10. Samples were frozen for analysis at a later date.

11. After thawing, samples were again filtered (Whatman 40) removing precipitate accumulated during freezing and passed through a membrane filter (.45 micron) before injection.

**Standard Preparation and Quantitation of Organic Acids**

Five aqueous organic acid standards covering a broad concentration range were prepared from stock solutions. Calibration curves were established to check for linearity and quantitations were based on peak height using the external standard technique described by Marsili et al. (1981).

**Free Fatty Acids Analysis**

Free fatty acids such as butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic were monitored by gas chromatography as described by Deeth et al. (1983). The
following were equipment and operating conditions utilized in this study: Perkin-Elmer Sigma 3b gas chromatography with flame ionization detector; Perkin-Elmer Sigma 15 computer console and integrator; injector and detector temperature of 230°C; glass column (3 m x 2 mm i.d.) packed with 10% SP-216-PS on 100/120 Supelcoport; temperature programs used were: initial temperature 110°C, heating rate 8°C/min to 195°C, holding time at final temperature (195°C) 22 min and nitrogen carrier gas at a flow rate of 55 ml/min.

Sample Preparation and Isolation of Free Fatty Acids

Hexane-diethyl ether extracts of product were obtained as follows: Cheddar cheese (1 g) chopped into small pieces was added to diethyl ether (5 ml). Then, .1 ml 4N-H₂SO₄ and granular anhydrous sodium sulphate (2.5 g) were added to absorb moisture. Extraction was carried out in 50 ml screw-capped polypropylene Sorvall centrifuge tubes. The mixture was thoroughly triturated and allowed to stand for at least one hour before hexane (5 ml) was added. A clear solution was obtained by brief centrifugation, approximately 2000 g/5 min at room temperature.

The total volume of hexane-diethyl ether solution of the product prepared as above was added carefully to a small polypropylene chromatography column (10 mm i.d.) containing neutral alumina (1 g). The solution was
passed through the column at approximately 3 ml/min and the eluate passed through the column a second time. This was followed by 2 x 5 ml of 1:1 v/v hexane-diethyl ether to remove all of the triglycerides. The total eluate was discarded. The alumina, with adsorbed fatty acids, was dried with vacuum applied to the column outlet and transferred to a small stoppered glass tube (50 mm x 8 mm i.d.). Diisopropyl ether containing 6% formic acid (1 ml) was added and mixed thoroughly with the alumina. The tube was centrifuged (2000 g/5 min) and a 3 ul aliquot of the supernatant injected into the gas chromatograph.

Alumina was chosen for this method because of its high affinity for acids and the simplicity of its use (Deeth et al., 1983). The use of formic acid to release the fatty acids from the alumina was found to be both efficient and convenient. Its inclusion also improved the shape of the fatty acid peaks in the gas chromatograms (Deeth et al., 1983). Formic acid showed a peak (x) in the chromatogram with a retention time shorter than that of butyric acid and which did not interfere with the chromatogram.

**Standard Preparation and Quantitation of Free Fatty Acids**

Between 4 and 56 mg of each fatty acid were weighed into a 100 ml volumetric flask. Formic acid (4.00 g) was added and diluted to the mark with diisopropyl ether. Five aqueous fatty acid standards covering a broad
concentration range were prepared from stock solutions. Calibration curves were established to check for linearity and quantitations were based on peak area using the external standard technique described by Deeth et al. (1983).

**Hull Test to Measure Proteolysis**

The Hull Test (1947) was used to determine differences in proteolysis due to psychrotrophic inoculation. This test includes incorporation of 10 g of cheese with 99 ml of 2% sodium citrate blank and blended for one minute. Ten milliliters of trichloroacetic acid (TCA .72 N) were added to 5.0 ml of the blended mixture to stop any further enzymatic activity and block further protein degradation. After addition of 1.0 ml distilled water, samples were mixed thoroughly and allowed to react for at least 10 min. Mixtures were then filtered through Whatman No. 40 filter paper and collected in clean dry test tubes. Four milliliters of the filtrate were transferred to a clean dry 50 ml Erlenmeyer flask. To this was added sodium carbonate-sodium tetraphosphate reagent (10 ml) with continuous agitation. Finally, 3.0 ml phenol reagent were added to develop the blue color. An additional 5 min was allowed for full color development. A Bausch and Lomb Spectronic 20 spectrophotometer was adjusted to zero with a closed lens and to 100% transmission with a 5 ml sample of distilled water
which had been treated exactly as the samples. Color intensity of the samples was then measured at 650 nm. Percent transmittance was then converted to micrograms of tyrosine per 5 ml sample with a standard curve plotting percent transmission as the X axis and micrograms of tyrosine as the Y axis.

**Flavor, Body and Texture Evaluation**

Cheddar cheese from each vat was subjected to sensory evaluation by a four-member trained panel at 5, 30, 60 and 180. Modified ADSA Cheddar cheese scorecard (Angevine et al., 1958) was used to score the flavor (1-10 scale), while body and texture were both scored with 1-5 scales. A flavor score of less than 5 was regarded as unacceptable (poor), 5-6 fair, 7-8 good and 9-10 excellent.

**Calculation of Cheddar Cheese Yield**

Actual cheese yield was determined by weighing each individual block of cheese after pressing (18 h). Theoretical cheese yield was calculated by the Van Slyke (1894) cheese yield formula:

\[
\frac{(0.93 \text{ fat} + \text{casein} - 0.1) \times 1.09}{100 - \text{Moisture percent}} = \text{cheese yield (lb. cheese from 100 lb. 4% fat milk)}
\]

Theoretical yields were calculated on a desired cheese moisture content of 39%.
Determination of Protein, Fat, Moisture, pH and Salt

The protein content of the psychrotrophic contaminated raw milk after 48 h incubation at 7°C was determined by Kjeldahl method as described by William et al. (1975). Fat contents of raw milk and Cheddar cheese were determined using Babcock method (Atherton and Newlander, 1977). Moisture percent of the cheese was determined using a vacuum oven. Salt content and pH of the cheese were determined following methods described by American Public Health Association (1978).

Statistical Analysis

The results of chemical, microbiological and flavor determinations were analyzed by the Analysis of Variance (ANOV) Technique in a randomized block design, blocked on batches of cheese, with a split plot in time arrangement treatment (Steel and Torrie, 1960). The F test was used to determine if significant differences existed among sources of variation. When differences were indicated, means were separated using Duncan's Multiple Range Test to determine where differences occurred (Steel and Torrie, 1960).
MATERIALS AND METHODS

Part II

The second phase of this research concentrated on the effects of psychrotrophic and coliform post-pasteurization contamination, high moisture and low activity starter on Cheddar cheese quality. Cheddar cheese preparation, as well as all tests performed, were the same as described in Part I of this manuscript.

The only requirement stipulated by the design of the experiment for the preparation of high moisture cheese was a milling acidity of 0.43% lactic acid and a moisture content of cheese of 40.5%.

Treatment Preparation

Grade A whole raw milk was pasteurized at 71.7°C for 15 sec (HTST) and separated for treatment as follows: control, psychrotroph at either 10,000 or 100,000/ml, and coliform at 1000/ml. In addition, high moisture cheese was made. Finally, a starter with low activity was used to make one cheese set. Tests performed were the same as described in Part I of this manuscript. Cheeses were stored at 7°C and sampled on days 0, 5, 30, 60 and 180. A schematic diagram of treatment preparation is depicted in Figure 8, and testing is outlined in Table 1.
Raw milk

\[ \rightarrow \]

\[ \rightarrow \]

Pasteurized 71.7°C/15 sec

\[ \rightarrow \]

\[ \rightarrow \]

Treatments

A. Added *Pseudomonas fluorescens* 27
   1. 10,000/ml
   2. 100,000/ml

B. Added Coliform (*Enterobacter aerogenes*)
   1. 1000/ml

C. High moisture cheese 40.5%

D. Added starter with low activity

E. Control - no added psychrotrophs or coliform

\[ \rightarrow \]

\[ \rightarrow \]

Cheese manufacture

\[ \rightarrow \]

\[ \rightarrow \]

All cheeses incubated at 7°C and analyzed for microbiological, chemical and flavor at d 0, 5, 30, 60 and 180.

---

**Figure 8.** Schematic diagram of treatment preparation and testing for Part II.
Isolation and Identification of Enterobacter aerogenes

Prior to this study, Enterobacter aerogenes was isolated on Violet Red Bile (VRE) agar and identified using the Eosine Methylene Blue (EMB) agar and the Enterotube II as illustrated in Figure 9. Enterobacter aerogenes was chosen based on its frequency of occurrence in dairy products. A schematic diagram of identification of Pseudomonas fluorescens and Enterobacter aerogenes is outlined in Figure 3. Enterotube II permits the inoculation of all media and the subsequent performance of 15 standard biochemical tests. The numbers corresponding to the positive reactions were totaled and the composite number was then located in the coding manual to identify the organism as shown in Figure 9.

Coliform Inoculation

In this study, a method was developed making possible the inoculation of coliform bacteria in known quantities to the pasteurized milk. Several methods and procedures were tested resulting in the following procedure.

1. Seven Trypticase Soy Agar (TSA) slants were prepared in test tubes of equal inside diameter and slanted to the same angle providing equivalent surface area.
**Figure 9.** Enterotube II for Identification of *Enterobacter aerogenes*
2. A standard loop of a stock culture of *Enterobacter aerogenes* was used, aseptically transferred and spread over the entire surface of each slant (one loop per slant).

3. After incubation at 32°C for 24 h, each slant was washed with 1 ml of buffered solution. This removed growth from the surface of the slant, resulting in a turbid suspension at the base.

4. A 1 ml portion from each slant in step 3 was transferred to corresponding bottles containing 100 ml sterile milk. Milk was previously heat treated in flowing steam for 1 h and cooled to 21°C.

5. These samples were incubated at 32°C for 24 h, followed by plating on TSA.

6. Plates were incubated for 24 h at 32°C to obtain an average count.

7. After establishing the average count in step 6, steps 1 through 5 were conducted with two slants prior to inoculation of pasteurized milk.

8. Duplicate bottles of sterile milk containing the coliform bacteria in step 5 were combined in a sterile Whirl-Pack bag (Nasco, Inc., Ft. Adkisson, WI) immediately prior to inoculation.

9. Inoculation levels were calculated based on the average count in step 6.
10. Stock cultures of *Enterobacter aerogenes* were transferred every 3 mo on TSA slants to maintain cultural activity. In addition, culture purity was verified routinely at time of transfer. TSA plates were streaked and incubated at 32°C for 24 h. Isolated colonies were selected and identified by the Enterotube II method. The above procedure proved very effective in obtaining desired levels of *Enterobacter aerogenes* in treatment preparations.
CHAPTER IV
RESULTS AND DISCUSSION

Part I

The purpose of this research was to examine chemical, microbiological and flavor changes in Cheddar cheese during 6 mo aging at 7°C. The first part of this study concentrated on the effect of psychrotrophs in raw milk on Cheddar cheese quality. This problem was considered to be of commercial importance; therefore, every effort was made to follow commercial practices in the manufacture of this product.

Effect of Psychrotrophic Contamination on Flavor, Body and Texture of Cheddar Cheese

In this study, flavor evaluations were conducted on 5, 30, 60 and 180 d: The Analysis of Variance Technique (ANOV) was performed to partition sources of variation. The F test described by Steel and Torrie (1960) was used to determine if differences in sources of variation existed. If differences were found, means for both psychrotrophic bacteria levels and changes during storage were separated by Duncan's Multiple Range Test (Steel and Torrie, 1960).

Mean flavor scores for the control and high psychrotrophic inoculation level (Table 2) revealed that the
control cheese had a significantly superior flavor score than all the psychrotrophic treated cheeses. In addition, cheese treated with *P. fluorescens* P27 had significantly (P < .001) lower flavor score than *P. fluorescens* P103. Results shown in Table 14 indicate that *P. fluorescens* P27 had significantly (P < .001) higher proteolytic activity than *P. fluorescens* P103. The lower flavor score caused by *P. fluorescens* P27 could be attributed to its high proteolytic activity.

The predominant flavor criticisms in the cheese made from psychrotrophic contaminated raw milk were bitter and unclean after 60 d of refrigerated (7°C) storage (Table 3). By 180 d bitterness was detected in 38% of the cheeses inoculated with both medium and high levels of psychrotrophs; none of the control samples were criticized as bitter (Table 4). This indicates probable production of bitter peptides by the psychrotrophic bacteria. By 180 d unclean flavor was detected in 25% of the cheeses inoculated with both medium and high levels of psychrotrophs. On the same day, none of the control samples were criticized as unclean (Table 4). This occurrence supported data demonstrating that psychrotrophs at levels greater than 1,000,000/ml in the milk resulted in a bitter flavor during storage of the finished product even though numbers after processing were low (Patel and Blankenagel, 1972). Schormuller
Table 2. Effect of psychrotrophic contamination of raw milk on the flavor of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cheese</td>
<td>7.95 A*</td>
</tr>
<tr>
<td>P. fluorescens 103-10,000/ml</td>
<td>7.20 B</td>
</tr>
<tr>
<td>P. fluorescens 103-100,000/ml</td>
<td>6.90 BC</td>
</tr>
<tr>
<td>P. fluorescens 27-10,000/ml</td>
<td>6.65 C</td>
</tr>
<tr>
<td>P. fluorescens 27-100,000/ml</td>
<td>6.55 C</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P. < .001).

Table 3. Most frequently noted flavor criticisms over time for control and treated cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Psychrotroph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (d)</td>
<td>5</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>Flat</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
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<tr>
<td></td>
<td>Flat</td>
<td>Flat</td>
</tr>
<tr>
<td></td>
<td>Acid</td>
<td>Bitter</td>
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<td></td>
<td></td>
<td>Unclean</td>
</tr>
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<td></td>
<td>Bitter</td>
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<tr>
<td></td>
<td></td>
<td>Acid</td>
</tr>
<tr>
<td></td>
<td>Bitter</td>
<td>Sulfide</td>
</tr>
</tbody>
</table>


Table 4. Frequency distribution of flavor criticisms of control and psychrotroph treated cheese at 180d.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Samples</th>
<th>Flat (%)</th>
<th>Bitter (%)</th>
<th>Unclean (%)</th>
<th>Acid (%)</th>
<th>Sulfide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>5</td>
<td>1(20)</td>
<td>0</td>
<td>0</td>
<td>4(80)</td>
<td>0</td>
</tr>
<tr>
<td>II. P. fluorescens 103</td>
<td>10</td>
<td>2(10)</td>
<td>6(30)</td>
<td>5(25)</td>
<td>6(30)</td>
<td>1(5)</td>
</tr>
<tr>
<td>III. P. fluorescens 27</td>
<td>10</td>
<td>3(14)</td>
<td>8(38)</td>
<td>5(24)</td>
<td>0</td>
<td>5(24)</td>
</tr>
</tbody>
</table>

*Percent values based on total number of defects and not total number of samples.
More than one criticism could be used for each sample.
(1968) demonstrated that proteases and peptidases are involved in the flavor and texture development of both soft and hard types of cheeses. These results supported data from investigators (Mayerhofer et al., 1973) who isolated and characterized a protease from Pseudomonas fluorescens which retained 71% of its original activity after being heated at 71.4°C for 60 min. The enzyme hydrolyzed milk protein at 4°C. When a protease of P. fluorescens was added to milk for Cheddar cheese making and the milk then held for 12 h at 4°C prior to manufacture, flavor scores were significantly lower than those of control samples; bitterness occurred most frequently in cheese made with Pseudomonas fluorescens. Juffs (1974) reported off-flavor formation by pseudomonas proteinases in Australian Cheddar cheese. Adams, Barach and Speck (1975) and Driessen (1976) reported that added heat-resistant pseudomonas proteases caused rapid spoiling of sterile milk with the development of bitter flavor.

The excessive bitterness in some cheese is objectionable, and therefore of considerable economic significance. Excessive bitterness is influenced by the cheese making procedure and by factors such as strain of starter culture, rennet concentration, heat treatment of the milk, acidity or pH of cheese and salt concentration (Emmons et al., 1962).
Bitterness, a flavor defect of cheese and of other cultured dairy products, has been found to be due to the accumulation of bitter-tasting peptides (Raadsveld, 1953) which are formed during the normal breakdown of casein by rennet and by bacterial proteolytic enzymes (Stadhouders, 1959; Emmons, 1962a,b; Gordon and Speck, 1965b; Harwalkkar and Seitz, 1971; Harwalkkar and Elliott, 1971; Richardson and Creamer, 1973; Hamilton et al., 1974; Fryer, 1969; Stadhouders and Hup, 1974; Lawrence, 1976).

All the bitter compounds which have been extracted from cheese and other cultured dairy products have been identified as peptides originating from \( \alpha \)- or \( \beta \)-casein. These peptides are fragments split off the casein molecules by proteolytic enzymes present in the rennet extracts and in the starter bacteria (Jago, 1974). These peptides, which originated from \( \beta \)-casein, have special chemical properties which enable them to react with the taste buds at the back of the tongue to give the sensation of bitterness (Jago, 1974). However, the bacterial peptidases may also contribute to the formation of bitter peptides by reducing the size of peptides initially too large to give a bitter taste (Sullivan and Jago, 1972; Sullivan, Mou, Rood and Jago, 1973).

An alternative and possibly more realistic explanation for the off-flavor and lower flavor scores in cheeses treated with psychrotrophs is that the incidence
of lipolytic activity among the psychrotrophic Gram negative flora of the raw milk was significantly (P < .001) higher in strains of P. fluorescens P27 and P103 than in the control cheese as shown in Tables 26 and 27 (also in Figures 46, 47, 48 and 49). Pseudomonas lipase, carried over from stored raw milk into cheese, is a well-established source of off-flavors and has never been cited as a source of improvement even when progressive ripening was studied (Law, Sharp and Chapman, 1976; Cousin and Mart, 1977; Cogan, 1977). Cogan (1980) reviewed several studies that discussed the deteriorative effect of heat-resistant microbial lipases on cheese quality and he found that psychrotrophic bacteria produce most of the heat-resistant microbial lipases.

Mean body/texture scores for the control and high psychrotrophic inoculation level (Table 5) revealed that cheese made from psychrotrophic contaminated raw milk had significantly (P < .001) lower body/texture scores than the control. In addition, cheese treated with P. fluorescens P27 had significantly (P < .001) lower body/texture score than P. fluorescens P103. Results shown in Table 14 indicate that P. fluorescens P27 had significantly (P < .001) higher proteolytic activity than P. fluorescens P103. The predominant body/texture criticisms noted after 60 and 180 d were weak, open and gassy (Tables 6 and 7). These defects are often seen in
Table 5. Effect of psychrotrophic contamination of raw milk on the body of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Body Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.20 A*</td>
</tr>
<tr>
<td>2. <em>P. fluorescens</em> 103-10,000/ml</td>
<td>3.65 B</td>
</tr>
<tr>
<td>3. <em>P. fluorescens</em> 103-100,000/ml</td>
<td>3.60 B</td>
</tr>
<tr>
<td>4. <em>P. fluorescens</em> 27-100,000/ml</td>
<td>3.35 BC</td>
</tr>
<tr>
<td>5. <em>P. fluorescens</em> 27-10,000/ml</td>
<td>2.90 C</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
high moisture cheese. All cheeses made with psychrotrophic contaminated raw milk resulted in cheese with significantly (P < .001) higher moisture content than the control (Table 16). Yan et al. (1983) reported that moisture content increased for cheese made from raw milk held under prolonged storage at 7°C. Such raw milk may undergo excessive proteolysis which increases the water-binding capacity of the denatured milk proteins in the curd resulting in soft-bodied curd. Schormuller (1968) has demonstrated that proteases are involved in texture development of both soft and hard types of cheeses. The degradation of proteins by microbial proteases contributes to a softening of the cheese (Arun and Shahani, 1978). This occurrence supported data by others demonstrating that the enzyme-treated cheeses were softer-bodied and more brittle than untreated cheeses of the same age (Law et al., 1982).

Microbiological profile of Cheddar cheese at different aging times are shown in Tables 8 and 9. These profiles revealed that all the microbiological counts showed that lactobacilli (Figures 10 and 13), streptococci (Figures 10 and 12) and total aerobic counts (Figures 10 and 11) tend to decrease with time and were significantly (P < .001) lower at both 60 and 180 d. At 0 d both psychrotrophic (Figure 14) and coliform (Figure 15) counts were significantly (P < .001) higher than the
Table 6. Most frequently noted body/texture criticisms over time for control and psychrotrophic treated Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (d)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>30</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>1. Control</td>
<td>Open</td>
<td>Open</td>
<td>Open</td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
<td>Curdy</td>
<td>Open</td>
<td>Open</td>
</tr>
<tr>
<td>2. Psychrotroph</td>
<td>Weak</td>
<td>Weak</td>
<td>Weak</td>
<td>Weak</td>
</tr>
<tr>
<td></td>
<td>Open</td>
<td>Open</td>
<td>Open</td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
<td>Curdy</td>
<td>Curdy</td>
<td>Gassy</td>
</tr>
<tr>
<td></td>
<td>Crumbly</td>
<td>Gassy</td>
<td>Gassy</td>
<td>Gassy</td>
</tr>
<tr>
<td></td>
<td>Gassy</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Frequency distribution of body/texture criticisms of control and psychrotrophic treated Cheddar cheese at 180 d.

<table>
<thead>
<tr>
<th>Criticism</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Samples</td>
<td>Weak</td>
</tr>
<tr>
<td>1. Control</td>
<td>5</td>
</tr>
<tr>
<td>2. P. fluorescens P103</td>
<td>10</td>
</tr>
<tr>
<td>3. P. fluorescens P27</td>
<td>10</td>
</tr>
</tbody>
</table>

*Percent values based on total number of defects and not total number of samples. More than one criticism could be used for each sample.
Table 8. Standard plate count and streptococci count of Cheddar cheese at different aging times.

<table>
<thead>
<tr>
<th>Days</th>
<th>SPC</th>
<th>Streptococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>664,280,000 A</td>
<td>762,400,000 A</td>
</tr>
<tr>
<td>5</td>
<td>797,200,000 A</td>
<td>1,030,800,000 B</td>
</tr>
<tr>
<td>30</td>
<td>506,880,000 A</td>
<td>505,240,000 C</td>
</tr>
<tr>
<td>60</td>
<td>189,440,000 B</td>
<td>77,600,000 D</td>
</tr>
<tr>
<td>180</td>
<td>4,835,800 B</td>
<td>201,760 D</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).

Table 9. Coliform, psychrotrophic and lactobacilli counts of Cheddar cheese at different aging times.

<table>
<thead>
<tr>
<th>Days</th>
<th>Coliform</th>
<th>Psychrotrophs</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>985.88 A *</td>
<td>15,354 A</td>
<td>1,158,600,000 A</td>
</tr>
<tr>
<td>5</td>
<td>968.84 A</td>
<td>3,513 B</td>
<td>1,398,400,000 A</td>
</tr>
<tr>
<td>30</td>
<td>730.88 A</td>
<td>3,032 B</td>
<td>856,400,000 B</td>
</tr>
<tr>
<td>60</td>
<td>103.10 A</td>
<td>2,044 B</td>
<td>334,320,000 C</td>
</tr>
<tr>
<td>180</td>
<td>16.88 A</td>
<td>31 B</td>
<td>2,709,680 D</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P .001).
other days. The decline of these bacteria during storage of Cheddar cheese at 7°C could be related to the effect of acid that builds up due to the lactic acid producing bacteria. In addition, many of these Gram negative rods were injured because of the cooking temperature and salting. A more realistic explanation was offered by Law (1982) who concluded that bacterial growth in the Cheddar cheese was limited by the conditions of acidity, salt concentration and redox potential so that full, mature flavor might take up to 12 mo to develop.

Juffs and Babel (1975) have studied the inhibition of psychrotrophs due to the addition of a variety of commercial lactic cultures to milk. In the same study, it was revealed that the inhibition of psychrotrophs resulted in a reduction of growth rate rather than the reduction of initial psychrotrophic population.

Effect of Psychrotrophs on Cheddar Cheese Yield

Cheddar cheese yield is important to determine the price of cheese. Results shown in Table 10 indicate that the actual yield of the control cheese was based on the weight of each individual block of cheese after 18 h pressing. This yield (control) was significantly (P<.001) higher than three of the four cheeses that were made from milk containing added psychrotrophs. No significant differences were noted in all the theoretical
Figure 10. Microbiological profile of Cheddar cheese at different aging times.
Figure 11. Total bacterial count (SPC) of control cheese vs. cheese made from psychrotrophic contaminated raw milk at different aging times.
Figure 12. Streptococcal population of control cheese vs. cheese made from psychrotrophic contaminated raw milk at different aging times.
Figure 13. Lactobacilli counts of control cheese vs. cheese made from psychrotrophic contaminated raw milk at different aging times.
Figure 14. Psychrotrophic counts (CVT) of control cheese vs. cheese made from psychrotrophic contaminated raw milk at different aging times.
Figure 15. Coliform counts of control cheese vs. cheese made from psychrotrophic contaminated raw milk.
Table 10. Effect of psychrotrophic contamination of raw milk on the yield of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Yield-Weight</th>
<th>Mean Theoretical Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. P. fluorescens 103-10,000/ml</td>
<td>10.632 A</td>
<td>10.800 A</td>
</tr>
<tr>
<td>3. P. fluorescens 103-100,000/ml</td>
<td>10.50 B</td>
<td>10.812 A</td>
</tr>
<tr>
<td>4. P. fluorescens 27-10,000/ml</td>
<td>10.49 B</td>
<td>10.816 A</td>
</tr>
<tr>
<td>5. P. fluorescens 27-100,000/ml</td>
<td>10.40 B</td>
<td>10.834 A</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).
cheese yields that were calculated by Van Slyke's (1984) cheese yield formula.

Over all storage time at 7°C, the control cheese showed significantly (P < .001) less proteolysis than the cheese that was made from milk containing added psychrotrophs (Table 14). This observation supported data by Swart et al. (1983), who also reported that Cheddar cheese yield decreased as counts of psychrotrophs in raw milk increased on storage. Cheddar cheese yield reduction was correlated with both lipid and protein degradation. Cheddar cheese yield was not affected when grade A raw milk was stored at 4 or 7°C up to 6 and 4 d, respectively (Yan et al., 1983). A rapid decrease in yield was observed when bacterial counts were greater than 10^8 CFU/ml. Decrease in yield was related to increases in proteolysis of the raw milk and to increases in TCA-soluble nitrogen in whey (Yan et al., 1983). Larry (1984) reported that proteases elaborated by psychrotrophic bacteria are capable of damaging the casein micelle and, consequently, capable of reducing cheese yield.

Psychrotrophs have been reported to have proteolytic and lipolytic activity and to produce undesirable breakdown products in milk (Adams et al., 1976; DeBeukelar et al., 1977; Law, Sharp and Chapman, 1976; Nelson and Marshall, 1977). Several researchers (Cousin and Marth,
1977a,b,c; Law, Sharp and Chapman, 1976; Nelson and Marshall, 1977) have stated that breakdown products soluble in whey result in lower cheese yield. Allauddin et al. (1976) reported that Cheddar cheese yields tended to be lower because of high proteolytic activity of a protease from psychrotrophs. This finding was supported by Cousin and Marth (1977a,b,c) who observed a considerable increase in total nitrogen in whey from milk inoculated with psychrotrophs. Feuillat et al. (1976) showed that excessive amounts of N were lost into the whey of soft cheese made with heat-treated high-count milk (approximately $10^6$ psychrotrophs per milliliter). The projected loss of cheese yield represented by the whey N losses was about 5%, a figure of considerable economic significance.

Yates and Elliott (1977) calculated that the growth of unspecified proteolytic psychrotrophic bacteria in whole milk resulted in whey N losses equivalent to reductions of 2.75-4.60% in the protein available for cheese. The lower percentage losses were associated with bacterial populations of approximately $10^7$ CFU/ml (assuming the majority of the psychrotrophic flora to be proteolytic). Research has indicated that growth of proteolytic psychrotrophs during low temperature storage may decrease milk quality, which results in a decrease in cheese yield due to the degradation of milk proteins and
increased loss of nonprotein nitrogen in whey (Aylward et al., 1980).

The yield of direct-acid cheese manufactured from inoculated milk with psychrotrophic *Bacillus* and *Pseudomonas* isolates decreased as psychrotrophic inoculation level increased. Yield reduction resulted from both lipid and protein degradation, and accounted for approximately 45 and 55% of the dry matter loss, respectively. Fat losses were observed from decreased milk fat tests and increased acid degree values. Protein losses were observed from increased non-protein nitrogen and whey nitrogen values (Hicks et al., 1982).

Data revealed in Table 11 demonstrate that the control cheese had significantly \((P < .001)\) higher pH values (5.20) over all days of storage than the cheese made from milk containing added psychrotrophs (5.06-5.09). This result supported data by Cousin and Marth (1977a) who reported that the initial pH of control cheeses was 6.70 compared to 6.55 to 6.60 for cheeses made from milk cultured with psychrotrophic bacteria.

A significant \((P < .001)\) increase in pH value was observed between 0 (5.07) and 180 d (5.16) as shown in Table 12. This increase in pH could be related to increase in the buffering capacity that resulted from the increases in proteolytic activity in Cheddar cheese with aging at 7°C (Table 13).
Table 11. Effect of psychrotrophic contamination of raw milk on pH of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>5.20 A</td>
</tr>
<tr>
<td>2. <em>P. fluorescens</em> 103-10,000/ml</td>
<td>5.09 B</td>
</tr>
<tr>
<td>3. <em>P. fluorescens</em> 103-100,000/ml</td>
<td>5.08 B</td>
</tr>
<tr>
<td>4. <em>P. fluorescens</em> 27-10,000/ml</td>
<td>5.07 B</td>
</tr>
<tr>
<td>5. <em>P. fluorescens</em> 27-100,000/ml</td>
<td>5.05 B</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).

Table 12. Effect of aging on pH of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.0744 AB</td>
</tr>
<tr>
<td>5</td>
<td>5.0264 B</td>
</tr>
<tr>
<td>30</td>
<td>5.1016 AC</td>
</tr>
<tr>
<td>60</td>
<td>5.1336 CD</td>
</tr>
<tr>
<td>180</td>
<td>5.1624 D</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
Tyrosine Standard Curve

In measurement of proteolytic activity by the psychrotrophs, the Hull Test (Hull, 1947) was employed. Standard duplicate dilutions of tyrosine were prepared and percent transmittance at 650 nm was determined (Figure 16). The data fit a straight line very well as indicated by the regression coefficient of 0.9972.

The Effect of Aging and Added Psychrotrophs on Proteolysis and Quality of Cheddar Cheese

Upon statistical examination of tyrosine content, the ANOV technique revealed significant ($P < .001$) increases in tyrosine content between 0 and 180 d as shown in Table 13 and in Figures 17 and 18. This could account for some increases in proteolytic activity with aging. The effect of psychrotrophs on proteolysis was measured by Hull Test demonstrated in Table 14 and in Figure 18, where the control cheese showed significantly ($P < .001$) less proteolysis over all days of storage than cheeses made from milk containing added psychrotrophs. In addition, cheeses treated with *P. fluorescens* P27 had significantly ($P < .001$) higher proteolytic activity than cheeses treated with *P. fluorescens* P103.

This observation supported data by Yan et al. (1983), who also reported a marked increase in tyrosine equivalent (TE) in milk after 4 d storage at 7°C and after 6 d storage at 4°C. After storage for 8 d, TE had
Table 13. Effect of aging on the proteolytic activity of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean Tyrosine Content (μg/5ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>66.36 A*</td>
</tr>
<tr>
<td>5</td>
<td>93.06 B</td>
</tr>
<tr>
<td>30</td>
<td>150.86 C</td>
</tr>
<tr>
<td>60</td>
<td>211.31 D</td>
</tr>
<tr>
<td>180</td>
<td>350.43 E</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
TYROSINE (µg/5ml)

Figure 16. Standard Curve for the Determination of Tyrosine Concentration.
Figure 17. Effect of aging on the proteolytic activity of Cheddar cheese.
Figure 18. Proteolytic activity of control cheese vs. cheese made from psychrotrophic contaminated raw milk at different aging times.
Table 14. Effect of psychrotrophic contamination of raw milk on proteolytic activity of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Tyrosine Content (μg/5ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. P. fluorescens 27-100,000/ml</td>
<td>193.50 A*</td>
</tr>
<tr>
<td>2. P. fluorescens 27-10,000/ml</td>
<td>183.20 A</td>
</tr>
<tr>
<td>3. P. fluorescens 103-100,000/ml</td>
<td>177.50 B</td>
</tr>
<tr>
<td>4. P. fluorescens 103-10,000/ml</td>
<td>172.40 B</td>
</tr>
<tr>
<td>5. Control Cheese</td>
<td>145.50 C</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
increased 88.4% in milk stored at 4°C compared to 222% in milk stored at 7°C. The increase in TE was always associated with an increase in the psychrotrophic count. The increase in TE occurred 2 to 4 d after the psychrotrophic count exceeded $10^6$/ml (Yan et al., 1983). The contaminating flora of cheese (P. fluorescens), which during processing of raw milk is normally characterized by the raw milk flora of the cheese milk, significantly influences the cheese ripening, especially the proteolysis. The degradation of $\beta$-casein is of primary concern (Gallman et al., 1982).

Kinsella and Hwang (1976) pointed out that proteolysis is important for production of amino acids, which act as precursors of flavor compounds. The extent of protein breakdown has been used as a crude index of cheese ripening (Puham et al., 1960; Morris et al., 1963; Tawab et al., 1966; Abd El-Salam et al., 1973).

The percentages of bitterness increased during storage (Table 3) and higher percentages were observed with increasing psychrotrophic inoculation (Table 4). It was postulated that bitterness was due to chemical components other than tryptophane and tyrosine which are detected by the Hull Test.

A new spectrophotometric assay (Church et al., 1983) using O-phthaldialdehyde (OPA) may give a more accurate determination of proteolysis. Church et al. (1983)
stated that OPA forms adducts having similar high absorptivities with all but two α-amino groups (a weak reaction with cysteine and none with proline). According to these same authors, the high absorptivities with most α-amino groups give a better representation of proteolysis compared to the Hull Test which detects only tryptophane and tyrosine. It is important to note that the Hull Test and OPA methods are only indicators of proteolysis. The degree of proteolysis is not always directly proportional to the intensity of bitter flavor. This is due to the fact that the intensity of bitter flavor is often dependent on the types of peptides present, rather than absolute quantities (Harwalkar, 1984).

As expected, data revealed in Table 15 demonstrate a significant (P < .001) drop in moisture content of Cheddar cheese between 0 and 180 d. This result supported data by Barraquio et al. (1982) who demonstrated that moisture content of Cheddar cheese was significantly affected by ripening period. Moisture content decreased while salt content increased with age of cheese. A decrease in moisture content was noted as the cheese ripened and this could be attributed to continuous loss of water through evaporation and displacement of water as the salt penetrates (Geurts et al., 1974; Gomes, 1977).

Upon statistical examination of moisture content, the ANOV technique revealed significant (P < .001)
Table 15. Effect of aging on moisture content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean Moisture Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>38.27 A*</td>
</tr>
<tr>
<td>5</td>
<td>38.12 B</td>
</tr>
<tr>
<td>30</td>
<td>38.014 B</td>
</tr>
<tr>
<td>60</td>
<td>37.83 C</td>
</tr>
<tr>
<td>180</td>
<td>37.63 D</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
increases in moisture content due to added psychrotrophs as shown in Table 16. This occurrence supported data by Yan et al. (1983) who reported that the moisture content increased for cheese made from milk stored 8 d at 7°C. Raw milk held under prolonged storage may undergo excessive proteolysis which increases the waterbinding capacity of the denatured milk proteins in the curd. The soft-bodied curd also may be caused by its high moisture content. The average moisture content of the curd obtained was higher than that of commercial Cheddar cheese (45 vs. 39%) (Yan et al., 1983).

**Effect of Aging and Added Psychrotrophs to Raw Milk on Organic Acid Content and Flavor of Cheddar Cheese**

A consideration of the literature from the last 25 years shows that sophisticated analytical techniques (chiefly GC and MS) have revealed the presence of a wide range of potentially flavorful compounds in cheeses. These include the following categories: volatile and nonvolatile fatty acids; alcohols; esters; lactones; ketones; aldehydes; hydrocarbons; pyrazines; peptides; amino acids; amines; and sulphur compounds (Law, 1982).

Figure 19 shows HPLC chromatograms of aqueous organic acid standards while Figures 20, 21, 22, 23 and 24 show the HPLC chromatograms of organic acids in Cheddar cheese samples 0, 5, 30, 60 and 180 d, respectively.
Table 16. Effect of psychrotrophic contamination of raw milk on moisture content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Moisture Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>P. fluorescens</em> 103-10,000/ml</td>
<td>38.46 A*</td>
</tr>
<tr>
<td>2. <em>P. fluorescens</em> 27-10,000/ml</td>
<td>38.36 A</td>
</tr>
<tr>
<td>3. <em>P. fluorescens</em> 103-100,000/ml</td>
<td>38.35 A</td>
</tr>
<tr>
<td>4. <em>P. fluorescens</em> 27-100,000/ml</td>
<td>37.83 A</td>
</tr>
<tr>
<td>5. Control Cheese</td>
<td>36.85 B</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
Results of HPLC analysis shown in Tables 17 and 18, also graphically depicted in Figures 25 and 26, indicate significant (P < .001) increases of citric, pyruvic, lactic, formic, acetic and propionic acids with aging of the cheese. Only orotic acid decreased significantly (P < .001) with aging. These results were consistent with those of Marsili (1981) who also reported a decline in concentrations of orotic acid during storage of Cheddar cheese at 7°C.

These results supported data from previous investigators (Chen and Larson, 1971; Epie and Melachouris, 1977; Larson and Hegarty, 1979) who concluded orotic acid to be readily utilized by various bacteria used in the fermentation of dairy products. Cheddar cheese ripening showed a dramatic decrease in lactose during pressing with subsequent increases in lactic and citric acids. Further ripening yielded slight increases in lactic acid, stabilizing of citric acid, and trace development of other acids. Extraction and analysis of other varieties of cheese indicated additional acids including pyruvic, acetic, propionic and butyric acids. Lactic acid was the predominant acid in all cheeses analyzed (Irwin et al., 1984).

Results in Tables 19 and 20 revealed significantly (P < .001) higher levels of lactic and formic acids and lower levels of propionic acid in cheeses made from
Figure 19. HPLC chromatograms of aqueous organic acids standards.
Figure 20. HPLC chromatograms of organic acids in Cheddar cheese at 0 day of aging. The peak at 5 min. is primarily phosphates.
Figure 21. HPLC chromatograms of organic acids in Cheddar cheese at 5 days of aging. The peak at 5 min. is primarily phosphates.
Figure 22. HPLC chromatograms of organic acids in Cheddar cheese at 30 days of aging. The peak at 5 min. is primarily phosphates.
Figure 23. HPLC chromatograms of organic acids in Cheddar cheese at 60 days of aging. The peak at 5 min. is primarily phosphates.
Figure 24. HPLC chromatograms of organic acids in Cheddar cheese at six months of aging. The peak at 5 min. is primarily phosphates.

Retention time (min.)

1 Orotic  6 Acetic
2 Citric   7 Propionic
3 Pyruvic  8 Butyric
4 Lactic   9 Unknown
5 Formic   10 Unknown
Table 17. Effect of aging on the orotic, citric, pyruvic and lactic acid content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Orotic  (ppm)</th>
<th>Citric  (ppm)</th>
<th>Pyruvic  (ppm)</th>
<th>Lactic  (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.99 A*</td>
<td>1089.4 A</td>
<td>24.35 A</td>
<td>5950.7 A</td>
</tr>
<tr>
<td>5</td>
<td>13.10 B</td>
<td>1150.8 A</td>
<td>25.56 A</td>
<td>6487.4 A</td>
</tr>
<tr>
<td>30</td>
<td>9.80 C</td>
<td>1255 B</td>
<td>36.52 B</td>
<td>6967.4 C</td>
</tr>
<tr>
<td>60</td>
<td>6.36 D</td>
<td>1105.3 C</td>
<td>37.67 B</td>
<td>6608.5 B</td>
</tr>
<tr>
<td>180</td>
<td>3.95 E</td>
<td>1841 D</td>
<td>65.96 C</td>
<td>8626.4 D</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).

Table 18. Effect of aging on the formic, acetic and propionic acid content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Formic  (ppm)</th>
<th>Acetic  (ppm)</th>
<th>Propionic  (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>139.3 A*</td>
<td>100.3 A</td>
<td>465.4 A</td>
</tr>
<tr>
<td>5</td>
<td>161.40 A</td>
<td>132.8 B</td>
<td>522.3 B</td>
</tr>
<tr>
<td>30</td>
<td>212.3 B</td>
<td>176.5 C</td>
<td>617.95 C</td>
</tr>
<tr>
<td>60</td>
<td>266.54 C</td>
<td>239.2 D</td>
<td>679.7 D</td>
</tr>
<tr>
<td>180</td>
<td>239.54 B</td>
<td>319.44 E</td>
<td>751.95 E</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).
Figure 25. Effect of aging on propionic, formic, acetic, pyruvic and orotic acid content of Cheddar cheese.
Figure 26. Effect of aging on lactic and citric acid content of Cheddar cheese.
raw milk cultured with psychrotrophs than the control cheese. Although differences between the control and psychrotrophic contaminated cheeses were not statistically significant for the rest of organic acids, the control cheese had lower values than the psychrotrophic treated cheese for pyruvic acid content, and no trend was noted for acetic, citric and orotic acids (Table 20).

Fermentation and flavor development are achieved through the use of a starter culture containing the acid producing Streptococcus cremoris which ferments lactose thereby producing lactic, acetic, propionic, formic and pyruvic acids and traces of acetaldehyde, ethanol and carbon dioxide (Vedamuthu, 1977). Vasavada (1979) demonstrated that the flavor of cultured dairy products depends not only on the concentration of flavor compounds but also on the ratios in which these compounds are present. Marshall and Harmon (1978) and Zandstra and deVries (1977) utilized colorimetric analysis and suggested measurement of the pyruvate content in milk as an index of bacteriological quality. Milolajcik (1979) reported that the breakdown products produced by enzymes from psychrotrophic bacteria stimulate starter culture acid production, which in excess produces body and flavor defects.

Organic acids occur in dairy products as a result of the hydrolysis of butterfat (fatty acids), direct
Table 19 Effect of added psychrotrophs to raw milk on organic acid content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Organic Acid</th>
<th>Control Cheese ppm</th>
<th>Treated Cheese ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orotic</td>
<td>10.10</td>
<td>9.81-10.22 NS²</td>
</tr>
<tr>
<td>Citric</td>
<td>1280</td>
<td>1255-1322 NS</td>
</tr>
<tr>
<td>Pyruvic</td>
<td>33.10</td>
<td>35.0-43.10 NS</td>
</tr>
<tr>
<td>Lactic</td>
<td>6307</td>
<td>6938-7352 ***</td>
</tr>
<tr>
<td>Formic</td>
<td>173.6</td>
<td>198.5-231.0 ***</td>
</tr>
<tr>
<td>Acetic</td>
<td>185.20</td>
<td>179.71-213.6 NS</td>
</tr>
<tr>
<td>Propionic</td>
<td>742.8</td>
<td>494.6-656.1 ***</td>
</tr>
</tbody>
</table>

¹Range of treated cheese for all four psychrotrophs (P. fluorescens 27 and 103)

²NS = not significant,

***Significant (P < .001).

Table 20. Relationship of organic acids in control vs. treated cheese.

<table>
<thead>
<tr>
<th>No Trend Noted</th>
<th>Control Lower Than Treated Cheese</th>
<th>Control Higher Than Treated Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>Formic</td>
<td>Propionic</td>
</tr>
<tr>
<td>Citric</td>
<td>Lactic</td>
<td></td>
</tr>
<tr>
<td>Orotic</td>
<td>Pyruvic-NS</td>
<td></td>
</tr>
</tbody>
</table>
addition as acidulants (e.g., citric, lactic and propionic acids), normal bovine biochemical metabolic processes (e.g., citric, hipuric, uric, orotic and ascorbic acids), or bacterial growth (e.g., pyruvic, lactic, acetic and propionic acids). Quantitative determinations of these acids in dairy products is important to flavor studies, for nutritional reasons and as an indicator of bacterial activity (Marsili et al., 1981).

The major flavor compounds formed in Cheddar cheese from fermentation are lactic acid, diacetyl and acetic acid, while from maturation are amino acids, amines, volatile fatty acids, pentanone, hydrogen sulphide and methanethiol (Law, 1982).

The flavor of very young Cheddar cheese is similar to that of other internally-salted varieties made with mesophilic starters; it can be described as acid, slightly buttery and salty. The flavor compounds at this stage of manufacture are largely derived from the carbohydrate fermentation of the starter streptococci; these organisms are regarded by taxonomists as homofermentative with regard to lactose as they produce mainly lactic acid. However, they do possess alternative pathways of pyruvate metabolism which are expressed in the cheese vat to a degree which allows production of acetic acid, ethanol and acetaldehyde (Law, 1981 and 1982). The importance of these pathways was demonstrated by Czulak
and coworkers (1974) who showed that pyruvate dehydrogenase activity (a key step in diverting pyruvate to the flavorful metabolites) in starter streptococci was inhibited in cheese made with milk containing high levels of polyunsaturated fat. The cheeses were low in acetate, acetaldehyde and diacetyl and had rather bland flavor.

**Effect of Aging and Added Psychrotrophs to Raw Milk on Volatile Organic Chemicals and Flavor of Cheddar Cheese**

Other fat-derived flavor compounds implicated in Cheddar flavor include ketones (Law, Castanon and Sharp, 1976). Figure 27 shows the chromatograms for a headspace analysis of aqueous volatile organic standards while Figures 28, 29, 30, 31 and 32 show the chromatograms for a headspace analysis of Cheddar cheese samples at 0, 5, 30, 60 and 180 d, respectively.

Results of GCHS indicated in Table 21 and graphically demonstrated in Figure 33 revealed that 60 and 180 d had significantly (P < .001) higher acetone and 2-butanolone values and lower 2-pentanone values than the other days. Also 0 d had significantly lower values of ethanol than the other days.

Ketones such as acetone, 2-butanolone and 2-pentanone are reported by Marsili (1981) to be the breakdown products of butterfat. Manning (1977) indicated that 2-pentanone concentrations in normal cheese are good indices of cheese age but the compound is not necessarily
Figure 27. HSGC chromatograms of aqueous volatile organic standards.

1 = ACETONE
2 = 2-BUTANONE
3 = ETHANOL
4 = 2-PENTANONE
5 = 2-BUTANOL
6 = \textit{N}-PROFANOL
Figure 28. HSGC chromatograms of volatile organics in Cheddar cheese at 0 day of aging.

1 = ACETONE
2 = 2-BUTANONE
3 = ETHANOL
4 = 2-PENTANONE
5 = 2-BUTANOL
6 = N-PROpanol
Figure 29. HS-GC chromatograms of volatile organics in Cheddar cheese at 5 days of aging.

1 = ACETONE
2 = 2-BUTANONE
3 = ETHANOL
4 = 2-PENTANONE
5 = 2-BUTANOL
6 = N-PROPA NOL
Figure 30. HSGC chromatograms of volatile organics in Cheddar cheese at 30 days of aging.

1 = ACETONE
2 = 2-BUTANONE
3 = ETHANOL
4 = 2-PENTANONE
5 = 2-BUTANOL
6 = N-PROPAHOL
Figure 31. HSGC chromatograms of volatile organics in Cheddar cheese at 60 days of aging.
Figure 32. HSGC chromatograms of volatile organics in Cheddar cheese at 180 days of aging.
Table 21. Effect of aging on organic volatile content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Acetone ppm</th>
<th>2-Butanone ppm</th>
<th>Ethanol ppm</th>
<th>2-Pentanone ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.2992 A</td>
<td>0.2896 A</td>
<td>67.98 A</td>
<td>1.8824 A</td>
</tr>
<tr>
<td>5</td>
<td>0.5122 B</td>
<td>0.0535 B</td>
<td>88.38 B</td>
<td>2.2483 B</td>
</tr>
<tr>
<td>30</td>
<td>0.4672 B</td>
<td>0.0492 B</td>
<td>101.39 C</td>
<td>1.8940 A</td>
</tr>
<tr>
<td>60</td>
<td>0.7296 C</td>
<td>0.5108 C</td>
<td>84.51 B</td>
<td>1.3776 C</td>
</tr>
<tr>
<td>180</td>
<td>0.8468 C</td>
<td>0.5552 C</td>
<td>83.04 B</td>
<td>1.4176 C</td>
</tr>
</tbody>
</table>

1Means in a column not followed by the same letter differ significantly (P < .001).
Figure 33. Effect of aging on organic volatile content of Cheddar cheese.
involved in flavor. The compound 2-butanoic acid is never found at concentrations higher than its threshold (Kinsella, 1969) and tends to disappear as cheese ages (Keen et al., 1974).

Upon statistical examination of chemical components, the ANOVA technique revealed significant (P < .05) increases in concentration of propanol due to added psychrotrophs to raw milk as shown in Table 22. Results indicated in Table 22 revealed that control cheese had significantly (P < .001) higher ethanol value than the psychrotrophic contaminated cheese.

The methyl ketone, 2-butanoic acid, has been widely reported to be present in Cheddar cheese (Scarpellino and Kosikowski, 1958; Lawrence, 1963; Kroger and Patton, 1964). Day, Bassette and Keeney (1960) estimated the concentration of 2-butanoic acid to be 12.5 ppm in mature Cheddar cheese, the highest concentration of any methyl ketone present. However, Bills, Willits and Day (1966) found up to 19 ppm of 2-butanoic acid in 4 of 10 Cheddar cheeses chosen at random. Keen and Walker (1974) also observed wide variations in the concentrations of 2-butanoic acid even in Cheddar cheeses made using the same single-strain starter (Streptococcus cremoris AM2).

Scarpellino (1961) suggested that 2-butanoic acid contributes to the desirable flavor complex of Cheddar cheese whereas the reduction product, 2-butanol, is associated with off-flavor development. A pathway has been
Table 22. Effect of added psychrotrophs on organic volatiles of Cheddar cheese.

<table>
<thead>
<tr>
<th>Organic Volatiles</th>
<th>Control Cheese</th>
<th>Treated&lt;sup&gt;1&lt;/sup&gt; Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ppm-</td>
<td>-ppm-</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.7526</td>
<td>0.46580-0.5680 NS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>0.27739</td>
<td>0.2892-0.3180 NS</td>
</tr>
<tr>
<td>Ethanol</td>
<td>94.130</td>
<td>68.772-88.935 ***</td>
</tr>
<tr>
<td>2-Pentanone</td>
<td>1.8643</td>
<td>1.6176-1.8400 NS</td>
</tr>
<tr>
<td>Propanol</td>
<td>1.1620</td>
<td>1.275-1.285 *</td>
</tr>
</tbody>
</table>

<sup>1</sup>Range of treated cheese for all four psychrotrophs (<em>P. fluorescens</em> 27 and 103)

<sup>2</sup>*Significant (<em>P</em> < .05)

NS = Not significant

***Significant (<em>P</em> < .001)
proposed for the formation of 2-butanone from 2,3-butylene glycol (Scarpellino and Kosikowski, 1962).

Keen and Walker (1974) suggested that an adventitious nonstarter bacterium in the Cheddar cheese may be responsible for the formation of 2-butanone.

The fruity defect is relatively common in Cheddar cheese and although it can be pleasant at low intensities it usually becomes unpleasantly strong in mature cheese. The defect occurs in cheeses which contain high ethanol levels (147-1527 ppm) (McGugan et al., 1975). Esterase activity in the cheese catalyses the reaction between volatile fatty acids and ethanol to produce esters whose flavor/aroma is reminiscent of pear drops. Ethyl butyrate and ethyl hexanoate (caproate) appear to be the main fruity esters in Cheddar cheese (McGugan et al., 1975; Bills et al., 1965). Manning (1979) reported that the high concentration of ethanol and \( \text{H}_2\text{S} \) found in cheese headspace have been related to the flavor defects "fruity" and "sulfide" respectively. The available microbiological evidence suggests that the fruity defect can be caused by the starter streptococci themselves, by heterofermentative nonstarter lactic acid bacteria, or by enzymes from psychrotrophic bacteria (Law, 1982).

Results indicated in Table 23 revealed a brief summary about the comparison of cheese made from psychrotroph-contaminated raw milk and control cheese.
Effect of Aging and Added Psychrotrophs on Free Fatty Acid Content and Flavor of Cheddar Cheese

Although free fatty acids (FFA) have been established as important flavor compounds in several of the aged cheese varieties with more pronounced flavors, only limited numbers of studies dealing with the definitive quantitative aspects of FFA in cheeses have appeared. The absence of an accurate, routine analytical method for quantification of individual major FFA in cheeses has hampered progress of definition of the role of FFA in cheeses (Aston et al., 1982).

The free fatty acids in cheese are derived from two major sources: (1) breakdown of the fat by lipolysis and (2) metabolism of carbohydrates and amino acids by bacteria. The bulk of evidence indicates that lipolysis is the principal contributor of free fatty acids of chain length C₄ or greater (Foda et al., 1974; Dulley and Grieve, 1974). Milk lipases have been shown to be more active than starter lipases in Cheddar (Reiter and Sharpe, 1971). They seem to hydrolyze the fat selectively and to be able to attack triglycerides, while lactic streptococci lipases seem to be active mainly on mono- and diglycerides (Stadhouders and Veringa, 1973).

Several workers (e.g., Pinheiro, Liska and Parmelee, 1965; Koshonti and Sjostrom, 1970; Driessen and Stadhouders, 1971; Law, Sharp and Chapman, 1976; Moskowitz, 1980) have shown that many extracellular
Table 23. Comparison of cheese made from psychrotroph-contaminated raw milk and control cheese.

<table>
<thead>
<tr>
<th>Higher than Control</th>
<th>Lower than Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate (NS)*</td>
<td>Acetate (P &lt; .001)</td>
</tr>
<tr>
<td>2-Butanone (NS)</td>
<td>2-Pentanone (NS)</td>
</tr>
<tr>
<td>Proteolytic (P &lt; .001)</td>
<td>Propionate (P &lt; .001)</td>
</tr>
<tr>
<td>Propanol (P &lt; .05)</td>
<td>pH (P &lt; .001)</td>
</tr>
<tr>
<td>Lactate (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>Formate (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>Moisture (P &lt; .001)</td>
<td></td>
</tr>
</tbody>
</table>

*Significance level in parentheses.
lipases from psychrotrophic raw milk bacteria (Pseudomonas spp) are resistant to pasteurization temperatures which kill the bacteria themselves. These enzymes are carried into the cheese and, in excess, could cause excessive lipolysis and rancidity in Cheddar cheese.

Figure 34 shows the gas chromatograms of a standard mixture of free fatty acids while Figures 35, 36, 37, 38 and 39 show the gas chromatograms of free fatty acids from 0, 5, 30, 60 and 180 d Cheddar cheese, respectively.

Data shown in Tables 24 and 25 of gas chromatograph analysis over all days of storage indicated significant (to at least P < .05) increases of butyric, caproic, caprylic, capric, lauric, myristic, palmitic and oleic acids with aging of the cheese (Figures 40, 41, 42 and 43). There were no significant increases of stearic and linoleic acids with aging of the cheese (Table 25). These observations supported data by Ohren and Tuckey (1969), who observed that high levels of free fatty acids (FFA) developed during maturation in cheeses made from heat-treated milk if levels of organisms were high in the raw milk, indicating that surviving bacterial lipases were also active in cheese.

Volatile fatty acids other than acetic increase during Cheddar cheese maturation due to the weak esterase
Figure 34. Gas chromatogram of a standard mixture of free fatty acids.
Figure 35. Gas chromatogram of free fatty acids from a 0-day-old Cheddar cheese.
Figure 36. Gas chromatograph of free fatty acids from a 5-day-old Cheddar cheese.
Figure 37. Gas chromatogram of free fatty acids from a 30-day-old Cheddar cheese.
Figure 38. Gas chromatogram of free fatty acids from a 2-month-old Cheddar cheese.
Figure 39. Gas chromatogram of free fatty acids from a 6-month-old Cheddar cheese.
Table 24. Effect of aging on free fatty acid (C4-C14) content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Butyric C4 -ppm-</th>
<th>Caproic C6 -ppm-</th>
<th>Caprylic C8 -ppm-</th>
<th>Capric C10 -ppm-</th>
<th>Lauric C12 -ppm-</th>
<th>Myristic C14 -ppm-</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24.902 A*</td>
<td>15.234 A**</td>
<td>12.062 A**</td>
<td>28.477 A*</td>
<td>37.907 A**</td>
<td>111.27 A*</td>
</tr>
<tr>
<td>5</td>
<td>34.745 B</td>
<td>18.188 AB</td>
<td>13.543 AB</td>
<td>32.068 BC</td>
<td>42.381 BC</td>
<td>124.42 AB</td>
</tr>
<tr>
<td>30</td>
<td>36.938 B</td>
<td>19.168 B</td>
<td>13.858 B</td>
<td>33.236 C</td>
<td>44.510 C</td>
<td>132.01 BC</td>
</tr>
<tr>
<td>60</td>
<td>33.246 B</td>
<td>16.642 AB</td>
<td>11.960 A</td>
<td>29.847 AB</td>
<td>39.601 AB</td>
<td>118.35 AB</td>
</tr>
<tr>
<td>180</td>
<td>39.181 B</td>
<td>19.557 B</td>
<td>14.108 B</td>
<td>34.076 C</td>
<td>42.717 CB</td>
<td>142.88 C</td>
</tr>
</tbody>
</table>

* Means in a column not followed by the same letter differ significantly (P < .01).
**Means in a column not followed by the same letter differ significantly (P < .05).
Table 25. Effect of aging on free fatty acid (C16-C18:2) content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Palmitic (C16 ppm)</th>
<th>Stearic (C18 ppm)</th>
<th>Oleic (C18:1 ppm)</th>
<th>Linoleic (C18:2 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>378.00 A*</td>
<td>235.45 A</td>
<td>26.325 A*</td>
<td>43.383 AB</td>
</tr>
<tr>
<td>5</td>
<td>426.24 AB</td>
<td>251.69 A</td>
<td>292.67 AB</td>
<td>46.83 AB</td>
</tr>
<tr>
<td>30</td>
<td>444.99 B</td>
<td>268.84 A</td>
<td>298.97 AB</td>
<td>45.399 AB</td>
</tr>
<tr>
<td>60</td>
<td>383.95 A</td>
<td>228.76 A</td>
<td>264.26 A</td>
<td>40.967 B</td>
</tr>
<tr>
<td>180</td>
<td>445.95 B</td>
<td>256.11 A</td>
<td>314.42 B</td>
<td>51.837 A</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .05).
Figure 40. Effect of aging on free fatty acid (C6, C8 and C18:3) content of Cheddar cheese.
Figure 41. Effect of aging on free fatty acid (C4, C10, C12 and C18:2) content of Cheddar cheese.
Figure 42. Effect of aging on free fatty acid (C14) content of Cheddar cheese.
Figure 43. Effect of aging on free fatty acid (C16, C18 and C18:1) content of Cheddar cheese.
and lipase activities of the milk flora and the starter bacteria (Stadhouders and Veringa, 1973). Although these volatile fatty acids are included in many synthetic cheese flavor formulations (Henning, 1970; Ney et al., 1972), evidence for their contribution to typical Cheddar cheese aroma/flavor is equivocal and contradictory. Generally, young Cheddar cheese contains low FFA, and aged, desirably-flavored Cheddar cheese has intermediate concentrations of individual FFA. However, when conditions permit development of more FFA in Cheddar, rancid off-flavors are readily apparent. This is specifically true for butyric acid (Woo et al., 1984).

Although butyric acid concentration (Table 26) had no significant difference between the control and the psychrotrophic treated cheese, still the cheese made from milk cultured with psychrotrophs revealed higher butyric acid value than the control cheese. Data shown in Table 26 of gas chromatograph analysis for free fatty acids over all days of storage indicated that the control cheese had significantly (P < .05) lower caproic, caprylic, capric, lauric, myristic, palmitic, stearic and oleic acid values than the cheese made from milk cultured with psychrotroph. In addition, by 180 d, significantly (P < .05) higher linolenic acid concentrations were noted in Cheddar cheese made from milk cultured with psychrotroph than the control cheese (Table 27).
Table 26. Effect of added psychrotrophs to raw milk on free fatty acid content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Free Fatty Acid</th>
<th>Control Cheese (ppm)</th>
<th>Treated Cheese (ppm)</th>
<th>Range</th>
<th>( \bar{X} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_4 )</td>
<td>28.602</td>
<td>25.711-41.79</td>
<td></td>
<td>34.00 NS²</td>
</tr>
<tr>
<td>( C_6 )</td>
<td>12.570</td>
<td>15.58-23.23</td>
<td></td>
<td>19.005 ***</td>
</tr>
<tr>
<td>( C_8 )</td>
<td>9.784</td>
<td>11.951-15.90</td>
<td></td>
<td>9.962 ***</td>
</tr>
<tr>
<td>( C_{10} )</td>
<td>23.428</td>
<td>28.40-37.09</td>
<td></td>
<td>33.569 ***</td>
</tr>
<tr>
<td>( C_{12} )</td>
<td>29.990</td>
<td>38.28-49.43</td>
<td></td>
<td>44.281 ***</td>
</tr>
<tr>
<td>( C_{14} )</td>
<td>104.11</td>
<td>112.30-141.20</td>
<td></td>
<td>131.202 ***</td>
</tr>
<tr>
<td>( C_{16} )</td>
<td>336.95</td>
<td>385.10-459.02</td>
<td></td>
<td>435.545 ***</td>
</tr>
<tr>
<td>( C_{18} )</td>
<td>200.88</td>
<td>237.28-276.00</td>
<td></td>
<td>259.99 *</td>
</tr>
<tr>
<td>( C_{18:1} )</td>
<td>231.76</td>
<td>270.20-324.47</td>
<td></td>
<td>300.45 ***</td>
</tr>
<tr>
<td>( C_{18:2} )</td>
<td>38.845</td>
<td>42.521-51.023</td>
<td></td>
<td>47.393 *</td>
</tr>
</tbody>
</table>

¹Range of treated cheese for all four psychrotrophs (P. fluorescens 27 and 103).

²Not significant (P > .05),

***Highly significant (P < .001),

* Significant (P < .05).
These observations supported data from previous investigators (Reiter, Fryer and Sharp, 1965 and 1966; Sharp, 1972) who concluded that although psychrotrophic gram negative rods, inoculated into low count milks and stored at low temperatures were eliminated by pasteurization, their lipolytic activity survived the process and gave rise to rancid flavored Cheddar cheeses containing high levels of free butyric and higher fatty acids. The highest incidence of lipolytic activity among the psychrotrophic gram negative flora of commercial raw milk was found in strains of *Pseudomonas fluorescens* (Law, Sharp and Chapman, 1976) and *Pseudomonas fluorescens* 27 (P 27) (Christen et al., 1980 and 1984). Cheeses made from milks in which mixed strains of lipolytic gram negative rods (GNR) had been allowed to multiply to greater than 10⁷ CFU/ml became rancid after four months even though the GNR had been killed by pasteurization. A single strain of *Pseudomonas fluorescens* (ARII) produced the same effect after only two months under similar conditions. The rancidity was characterized by a soapy off-flavor in cheeses containing free fatty acid concentrations from three to ten times higher than those in control cheeses made from stored milks with low GNR counts.

Data revealed in Table 28 demonstrate a significant (P < .001) negative correlation between the short chain
Table 27. Effect of added psychrotrophs to raw milk on linolenic (C18:3) content of Cheddar cheese.

<table>
<thead>
<tr>
<th></th>
<th>0 ppm</th>
<th>5 ppm</th>
<th>30 ppm</th>
<th>60 ppm</th>
<th>180 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>0 A*</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>16 A</td>
</tr>
<tr>
<td>2. P. fluorescens 10^3-10,000/ml</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 B</td>
</tr>
<tr>
<td>3. P. fluorescens 10^3-100,000/ml</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>17.92 C</td>
</tr>
<tr>
<td>4. P. fluorescens 27-10,000/ml</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>15.85 A</td>
</tr>
<tr>
<td>5. P. fluorescens 27-100,000/ml</td>
<td>0 A</td>
<td>0 A</td>
<td>16.78 B</td>
<td>16.18 B</td>
<td>18.715 D</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .05).
fatty acids at 30 d and cheese flavor at 180 d. Also indicated as significant (P < .01) was the negative correlation between lauric, myristic, palmitic and oleic acids at 30 d and cheese flavor at 180 d. This occurrence supported data by Patton (1963) who claimed that volatile fatty acids (C₂-C₈) were the "backbone" of Cheddar aroma because blocking agents for carboxylic functional groups impaired the aroma of cheese fat distillates. However, Manning and Price (1977) showed that the removal of volatile fatty acids from Cheddar cheese headspace did not affect its aroma at all and concluded that these acids were only important in the background taste of the cheese.

Nakae and Elliott (1965) demonstrated that the FFA from acetic to caproic were produced from casein hydrolysates by lactic acid bacteria. Acetic acid can be produced from citrate, lactose and amino acids (Fryer, 1969). Farrer and Weeks (1970) attributed Cheddar cheese flavor to one or more of the following compounds: acetic, butyric, caproic, capric acids, methional, 3-mercaptопropionic acid. The free fatty acids butyric, caproic, caprylic and capric were included in a patent specification submitted by Henning (1970) which was claimed to impart Cheddar cheese flavor to foodstuffs. Studies with enzyme-modified cheese suggest that increased free volatile fatty acids in lipase-treated
Table 28. Correlation of free fatty acids at 30 d with flavor at 180 d storage at 7°C.

<table>
<thead>
<tr>
<th>Free Fatty Acids</th>
<th>Correlation Coefficients</th>
<th>N</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric C4</td>
<td></td>
<td>15</td>
<td>-0.67**</td>
</tr>
<tr>
<td>Caproic C6</td>
<td></td>
<td>15</td>
<td>-0.82***</td>
</tr>
<tr>
<td>Caprylic C8</td>
<td></td>
<td>15</td>
<td>-0.79***</td>
</tr>
<tr>
<td>Capric C10</td>
<td></td>
<td>15</td>
<td>-0.77***</td>
</tr>
<tr>
<td>Lauric C12</td>
<td></td>
<td>15</td>
<td>-0.73**</td>
</tr>
<tr>
<td>Myristic C14</td>
<td></td>
<td>15</td>
<td>-0.71**</td>
</tr>
<tr>
<td>Palmitic C16</td>
<td></td>
<td>15</td>
<td>-0.68**</td>
</tr>
<tr>
<td>Oleic C18:1</td>
<td></td>
<td>15</td>
<td>-0.68**</td>
</tr>
</tbody>
</table>

** P < .01
***P < .001
American Cheddar increase its flavor intensity, provided that high rancidity-inducing lipase levels are avoided (Kosikowski and Iwasaki, 1975; Sood and Kosikowski, 1979).

Harper, Wang and Kristoffersen (1979) showed that free fatty acids from C₄ to C₁₀ were produced in vegetable fat modified Cheddar cheese, but their levels were significantly lower than those in the milk fat cheeses. Cheddar cheese manufactured with vegetable or mineral lipids appear not to develop characteristic flavor (Foda et al., 1974; Harper, Wang and Kristoffersen, 1979). Some apparently fine-flavored Cheddar cheeses have low concentration of the short-chain FFA (Woo et al., 1982). Free fatty acids derived from milk fat generally believed to contribute to the flavor of most aged cheese (Badings et al., 1980; Law, 1982; Adda et al., 1982). Hydrolytic rancidity flavor defects in Swiss, Brick and Cheddar cheeses were signaled by measurement of high concentrations of individual short-chain free fatty acids (Woo et al., 1984).

Data revealed in Figure 44 demonstrates a significant (P < .05) difference in myristic and linolenic acid concentrations between 30 and 180 d for the control cheese, while Figure 45 indicates a significant (P < .05) difference in linoleic acid concentration between 30 and 180 d for the cheese that was made from milk cultured with psychrotrophs.
Figure 44. Free fatty acid profile of control Cheddar cheese at 30 days versus 180 days of aging.
Figure 45. Free fatty acid profile of Cheddar cheese made from psychrotrophic (Strain 27) contaminated raw milk at 30 days versus 180 days.
Data revealed in Figures 46 and 48 demonstrate significantly (P < .05) higher concentrations of short and long chain fatty acids at 30 d aging in Cheddar cheese made from psychrotrophic contaminated raw milk than the control cheese. Also, Figures 47 and 49 indicate significantly (at least P < .05) higher concentrations of C4, C6, C8, C10, C12, C18:1 and C18:2 at 180 d aging in Cheddar cheese made from psychrotrophic contaminated raw milk than the control cheese.

These observations supported data from previous investigators (Deeth and Fitz-Gerald, 1975; Law, Sharp and Chapman, 1976) who concluded that psychrotrophic bacteria that possess heat stable lipases are also potential contributors to free fatty acid production during cheese ripening. Activity of microbial lipases is the predominant source of excessive lipolysis in cheese (Kuzdzal-Savoie, 1980).

Cogan (1980) reviewed several studies that discussed the deteriorative effect of heat-resistant microbial lipases on cheese quality and he found that psychrotrophic bacteria produce most of the heat-resistant microbial lipases.

The fat globule membrane (FGM) is an additional source of lipids (in particular, phospholipids), and its possible contribution to cheese flavor has not been considered previously, although recent reports have dealt
Figure 46. Free fatty acid profile of Cheddar cheese made from psychrotrophic (Strain 27) contaminated raw milk versus control at 30 days of aging.
Figure 47. Free fatty acid profile of Cheddar cheese made from psychrotrophic (Strain 27) contaminated raw milk versus control at 180 days of aging.
Figure 48. Free fatty acid profile of Cheddar cheese made from psychrotrophic (Strains 27 and 103) contaminated raw milk versus control at 30 days of aging.
Figure 49. Free fatty acid profile of Cheddar cheese made from psychrotrophic (Strain 27 and 103) contaminated raw milk versus control at 180 days of aging.
with isolation of phospholipids from cheese (Umemoto and Sato, 1970; Nakanishi and Kaya, 1971). Phospholipids in the cheeses decreased by 50 to 70% during the 6 mo ripening. The breakdown of phospholipids during ripening of Cheddar cheeses was attributed to phospholipases produced by the psychrotrophic bacteria where titers of free fatty acids increased at greater rates in raw milks incubated at 30°C with phospholipase C from Pseudomonas fluorescens (Chrisope and Marshall, 1976).

Results indicated in Table 29 revealed a significant (P < .001) negative correlation between the moisture content of Cheddar cheese at 0 d and cheese flavor at 180 d, i.e., the higher moisture at 0 d, the lower the quality of Cheddar cheese. Also, a significant (P < .001) positive correlation was noted between the pH and salt content of cheese at 0 and 30 d respectively and cheese flavor at 180 d. This supported data of previous investigators (Lawrence and Gilles, 1969) who concluded that the average salt-in-moisture levels of the non-bitter cheese were considerably higher than those in bitter cheese of the same acidity above and below pH level 4.95. The report of Fox and Walley (1971) helps to explain the role of salt in preventing bitterness in Cheddar cheese. Sullivan et al. (1971) demonstrated an effect of pH on the ability of "bitter" and "non-bitter" strains to remove bitterness from bitter peptides extracted from
Table 29. Correlation of cheese flavor* with moisture, salt and pH.

<table>
<thead>
<tr>
<th>Correlation of Flavor With</th>
<th>Day</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>0</td>
<td>-.76***</td>
</tr>
<tr>
<td>Salt</td>
<td>30</td>
<td>.70***</td>
</tr>
<tr>
<td>pH</td>
<td>0</td>
<td>.76***</td>
</tr>
</tbody>
</table>

* Flavor at 180 d.
***P < .001

Table 30. Regression of flavor* score on moisture, salt and pH.

<table>
<thead>
<tr>
<th>Mean Flavor Score at 180d</th>
<th>Equation</th>
<th>Mean X</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.72</td>
<td>(61.57)+(-1.43) (moisture)</td>
<td>38.27</td>
</tr>
<tr>
<td>6.71</td>
<td>(-7.711)+(4.90) (salt)</td>
<td>2.94</td>
</tr>
<tr>
<td>6.71</td>
<td>(-57.15)+(12.58) (pH)</td>
<td>5.07</td>
</tr>
</tbody>
</table>

*Flavor at 180d.
tryptic hydrolysates of casein. They reported that strain HP (bitter) of *Streptococcus cremoris* was unable to reduce the level of bitterness in the bitter peptide extract at pH values below 5.5, while strain MLI (non-bitter) could do so at all pH values above 4.5. This report provides evidence that pH is a determining factor in the degradation of bitter peptides by individual strains of starter.

**Prediction of Flavor**

The dairy industry has an immediate need for a rapid method to predict the flavor of finished product. Although recent advances have been made in the area of fluid milk to predict the shelf-life of finished products (Bishop and White, 1984), little is known about the predictability of flavor in cultured products.

Stepwise regression, parameters and their ratios on 0, 5 and 30 d were used to formulate regression equations to predict the flavor of Cheddar cheese at 180 d. Entering the moisture content at 0 d, streptococci counts at 5 d, pH at 5 d and acetic value at 0 d as shown (Table 31), a regression equation was formulated having a coefficient of determination ($R^2$) of 0.81. This means that 81% of the variation in flavor could be explained by these parameters.

Results obtained after free fatty acid analysis indicate that entering the pH at 0 d, lactobacilli counts
Table 31. Regression coefficients for equation\(^2\) to predict ripened Cheddar cheese flavor\(^1\) by analysis of young cheese.

<table>
<thead>
<tr>
<th>Variable</th>
<th>b Value</th>
<th>Prob.&gt;F</th>
<th>(R^2)</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>58.604</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X1 Moisture (day 0)</td>
<td>-1.386</td>
<td>0.0001</td>
<td>0.81</td>
<td>6.5</td>
</tr>
<tr>
<td>X2 Streptococci (day 5)</td>
<td>-1.446</td>
<td>0.0016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3 pH (day 5)</td>
<td>2.506</td>
<td>0.0009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X4 Acetic (day 0)</td>
<td>0.014</td>
<td>0.0590</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Flavor at 180 d.

\(^2\)Regression equation calculated as follows:
Flavor score = (58.604)+(-1.386)(X1)+(-1.446)(X2)+(2.506)(X3)+(0.014)(X4)
at 5 d, and caproic acid content at 30 d as shown (Table 32), a regression equation was formulated having a coefficient of determination ($R^2$) of 0.87. This means that 87% of the variation in flavor could be explained by these parameters. With the addition of coliform count at 0 d, Hull result at 5 d, acetic acid value at 30 d and oleic acid at 0 d to the pH at 0 d, lactobacilli count at 5 d and caproic acid value at 30 d (Table 33), the $R^2$ increased to .997 indicating almost perfect explanation of flavor differences. However, the quantitation of this many variables becomes very impractical when applied to a plant situation, e.g., the analysis required to quantitate these compounds is unwieldy for most dairy plant laboratories. Also, the application of the equation in Table 33 to future data sets would probably not be nearly as successful, due to changes in the b values.

In order to use these equations to predict Cheddar cheese flavor after 180 d, variables of an individual Cheddar cheese sample would be substituted in the equation on given days as shown (Table 33). After computation, the resulting flavor score would be that predicted for the cheese after 180 d storage. Rather than actually using the equations, one objective of this research was to identify the chemical components which best relate to the flavor of Cheddar cheese. After establishing this point, then further investigation of these components
Table 32. Regression coefficients for equation\textsuperscript{2} to predict ripened Cheddar cheese flavor\textsuperscript{1} by analysis of young cheese.

<table>
<thead>
<tr>
<th>Variable</th>
<th>b Value</th>
<th>Prob.&gt;F</th>
<th>R\textsuperscript{2}</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-9.680</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X\textsubscript{1} pH (day 0)</td>
<td>6.58</td>
<td>0.0048</td>
<td>0.87</td>
<td>6.5</td>
</tr>
<tr>
<td>X\textsubscript{2} Lactobacilli (day 5)</td>
<td>-1.61</td>
<td>0.0268</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X\textsubscript{3} Caproic Acid (day 30)</td>
<td>-0.13</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}Flavor at 180 d.

\textsuperscript{2}Regression equation calculated as follows:
Flavor score = -9.680 + (6.58)(X\textsubscript{1}) + (-1.61)(X\textsubscript{2}) + (-0.13)(X\textsubscript{3})
Table 33. Regression coefficients for equation\(^2\) to predict ripened Cheddar cheese flavor by analysis of young cheese including additional variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>b Value</th>
<th>Prob.&gt;F</th>
<th>(R^2)</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.469</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X1 Coliform (day 0)</td>
<td>0</td>
<td>0.173</td>
<td>0.0001</td>
<td>0.997 6.5</td>
</tr>
<tr>
<td>X2 pH (day 0)</td>
<td>0</td>
<td>7.513</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>X3 Lactobacilli (day 5)</td>
<td>5</td>
<td>-2.942</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>X4 Hull (day 5)</td>
<td>5</td>
<td>-0.039</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>X5 Acetic Acid (day 30)</td>
<td>30</td>
<td>0.004</td>
<td>0.0016</td>
<td></td>
</tr>
<tr>
<td>X6 Oleic Acid (day 0)</td>
<td>0</td>
<td>0.004</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>X7 Caproic Acid (day 30)</td>
<td>30</td>
<td>-0.162</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Flavor at 180 d.

\(^2\)Regression equation calculated as follows:
Flavor score = (-0.469) + (0.173)(X1) + (7.513)(X2) +
(-2.942)(X3) + (-0.039)(X4) + (0.004)(X5) +
(0.004)(X6) + (-0.162)(X7)
should yield more definite conclusions as to their actual importance in flavor predictions.

According to data revealed in Tables 32 and 33, the development of simple procedures to quantitate pH, lactobacilli and caproic acid along with coliform, Hull, acetic acid and oleic acid would be beneficial in estimating the potential flavor of Cheddar cheese at 180 d. However, the present results only indicate a relationship with flavor. Additional study of these compounds is needed to determine the repeatability of these relationships in order to assess their ability to predict the flavor.

Chemical Profiles of "Good" Versus "Bad" Cheddar Cheese

As previously shown (Table 2), the highest mean flavor score (good) over all days of storage was observed in control Cheddar cheese. In addition, the lowest mean flavor score (bad) was observed in Cheddar cheese made from contaminated raw milk with psychrotrophs. In order to establish profiles of "good" versus "bad" Cheddar cheese, mean chemical profiles on the days of storage were compared to note similarities and differences in composition. Results in Table 23 revealed significantly (P < .001) higher levels of proteolytic activity, lactate, formate, moisture and propanol (P < .05) in "bad" Cheddar cheese. In addition, there was significantly (P < .001) lower acetate, pH, propionate and 2-
pantanote (non-significant) in "bad" Cheddar cheese as opposed to "good" Cheddar cheese.

Although there was no significant (P > .05) difference in butyric acid concentration (Table 26) between the "good" (control cheese) and the "bad" (psychrotrophic treated cheese) cheese, the "bad" cheese revealed higher butyric acid value than the "good" cheese. Data shown in Table 26 for free fatty acids over all days of storage indicated significantly (P < .05) higher levels of caproic, caprylic, capric, lauric, myristic, palmitic, stearic and oleic acids in "bad" Cheddar cheese. In addition, by 180 d, significantly (P < .05) higher linolenic acid concentration was noted in "bad" Cheddar cheese (Table 27).

Mikolajcik (1979) concluded that the breakdown products from psychrotrophic bacterial lipases and proteases stimulate starter culture acid production, which in excess produces body and flavor defects. Ohren and Tuckey (1969) reported that Cheddar cheese with an acetate to fatty acid ratio between specified limits, developed good flavor after 180 d ripening. Overproduction of acetic acid can lead to vinegar-like off-flavors (Law and Castanon, 1976). Scarpellino (1961) concluded that 2-butanone contributes to the desirable flavor complex of Cheddar cheese whereas the reduction product, 2-butanol, is associated with off-flavor
development. Marsili (1981) reported that high levels of alcohol and acetone impairs cheese quality. Generally fatty acids have been acknowledged to contribute cheesiness in Cheddar cheese (Forss, 1979). However, there are claims that fatty acids are important because low fat or fat free cheeses did not develop flavor (Ohren and Tuckey, 1969). Woo et al. (1984) concluded that some apparently fine-flavored Cheddar cheese has low concentrations of the short chain free fatty acids. When conditions permit development of more FFA in Cheddar cheese, off-flavors are readily apparent (Woo et al., 1984).
RESULTS AND DISCUSSION

Part II

The first part of this research examined the effect of psychrotrophs in raw milk on Cheddar cheese quality. It was concluded that *P. fluorescens* P27 and P103 did have a detrimental effect on Cheddar cheese quality. Although these bacteria normally are destroyed by pasteurization, enzymes they produce are thermostable. These enzymes then cause deterioration of the Cheddar cheese quality. As a consequence of this earlier work, and since most of the keeping quality problems of pasteurized milk in the United States is a direct result of post-pasteurization contamination with *Pseudomonas* and *Enterobacter*, the second part of this research concentrated on the effect of post-pasteurization contamination and high moisture curd on Cheddar cheese quality.

**Effect of Psychrotrophic and Coliform Post-Pasteurization Contamination, and High Moisture Curd on the Flavor, Body and Texture of Cheddar Cheese**

The effect of psychrotrophs and coliform post-pasteurization contamination, high moisture and low activity starter on mean flavor scores are shown (Table 34). These results indicated consistently lower flavor scores in Cheddar cheese with increasing initial psychrotrophic inoculation. Mean separation of these
Table 34. Effect of psychrotrophic and coliform-post pasteurization contamination, high moisture and low activity starter on the flavor of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control Cheese</td>
<td>7.1667 A*</td>
</tr>
<tr>
<td>2. P. fluorescens 27-10,000/ml</td>
<td>6.3333 B</td>
</tr>
<tr>
<td>3. P. fluorescens 27-100,000/ml</td>
<td>6.2000 B</td>
</tr>
<tr>
<td>4. Coliform 1000/ml</td>
<td>6.4500 B</td>
</tr>
<tr>
<td>5. High Moisture</td>
<td>6.6667 A</td>
</tr>
<tr>
<td>6. Low Activity</td>
<td>6.0000 B</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .01).
data (Table 34) revealed that flavor scores of high psychrotrophic inoculation, coliform and low activity starter were significantly (P < .001) lower than the control over all days of storage. From these results, it was concluded that *P. fluorescens*, *Enterobacter aerogenes* and low activity starter did have a detrimental effect on Cheddar cheese flavor.

The predominant flavor criticisms in the cheese made from psychrotrophic and coliform post-pasteurization contaminated milk were bitter and unclean at 180 d (Table 35) while high moisture cheese exhibited fermented and whey taint flavor in addition to bitter and unclean flavor. By 180 d bitterness was detected in 40 and 55% of Cheddar cheese inoculated with psychrotrophs and coliforms, respectively. None (0%) of the control samples were criticized as bitter (Table 36). This indicates probable production of bitter peptides by the psychrotrophic and coliform bacteria. By 180 d unclean flavor was detected in 20-22% of Cheddar cheese inoculated with psychrotrophs and coliform while fermented and whey taint were detected in 22% of high moisture Cheddar cheese. None (0%) of the control samples were criticized as bitter, unclean, fermented and whey taint (Table 37).

As stated previously, the presence of psychrotrophic lipase, carried over from stored raw milk into
Table 35. Most frequently noted flavor vs. time for control and treated cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>Flat</td>
<td>Flat</td>
<td>Flat</td>
<td>Flat</td>
</tr>
<tr>
<td>2. Psychrotrophs</td>
<td>Flat</td>
<td>Flat</td>
<td>Flat</td>
<td>Bitter, unclean acid, rancid</td>
</tr>
<tr>
<td>3. Coliform</td>
<td>Flat</td>
<td>Flat</td>
<td>Flat</td>
<td>Bitter, unclean</td>
</tr>
<tr>
<td>4. High Moisture</td>
<td>Flat</td>
<td>Flat</td>
<td>Acid sulfide</td>
<td>Bitter, unclean acid, fermented whey taint</td>
</tr>
</tbody>
</table>
Table 36. Frequency distribution of flat, bitter, unclean and acid flavor criticisms of control and psychrotroph treated cheese at 180 d.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Samples</th>
<th>Flat</th>
<th>Bitter</th>
<th>Unclean</th>
<th>Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>3</td>
<td>3(100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. Psychrotroph</td>
<td>10</td>
<td>4(26.66)</td>
<td>6(40)</td>
<td>3(20)</td>
<td>1(6.66)</td>
</tr>
<tr>
<td>3. Coliform</td>
<td>5</td>
<td>2.222</td>
<td>5(55.56)</td>
<td>2(22.22)</td>
<td>0</td>
</tr>
<tr>
<td>4. High Moisture</td>
<td>5</td>
<td>0</td>
<td>2(22.22)</td>
<td>1(11.11)</td>
<td>2(22.22)</td>
</tr>
</tbody>
</table>

*Percent values based on total number of defects and not total number of samples.
More than one criticism could be used for each sample.

Table 37. Frequency distribution of sulfide, rancid, fermented and whey taint flavor criticisms of control and psychrotroph treated cheese at 180 d.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Samples</th>
<th>Sulfide</th>
<th>Rancid</th>
<th>Fermented</th>
<th>Taint</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. Psychrotroph</td>
<td>10</td>
<td>0</td>
<td>1(6.66)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. Coliform</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4. High Moisture</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2(22.22)</td>
<td>2(22.22)</td>
</tr>
</tbody>
</table>

*Percent values based on total number of defects and not total number of samples.
More than one criticism could be used for each sample.
cheese, is a well-established source of off-flavors and has never been cited as a source of cheese improvement (Law, Sharp and Chapman, 1976; Cousin and Marth, 1977; Cogan, 1977).

The incidence of lipolytic activity among the *P. fluorescens* P27 was found to have significantly (at least $P < .05$) higher concentrations of both short and long chain free fatty acids than the control cheese (Figures 67 and 68). Coliform post-pasteurization and high moisture Cheddar cheese exhibited significantly ($P < .05$) higher concentration of linolenic (C18:3) than the control cheese (Figures 72 and 73) at 30 and 180 d, respectively.

An alternative and possibly more realistic explanation for the off-flavor and lower flavor scores in cheeses treated with psychrotrophs, coliform and also in high moisture curd is the significantly ($P < .001$) higher proteolytic activity than the control cheese (Table 44 and Figure 57). These results supported data from previous investigators (Gallman et al., 1982) who concluded that the contaminating flora of cheese (*E. coli* and *P. fluorescens*) significantly influence the cheese ripening, especially the proteolysis. Of main concern is the degradation of B-casein. Cogan (1980) reviewed several studies that discussed the deteriorative effect of heat-resistant psychrotrophic lipases and proteases on Cheddar cheese quality.
Mean body/texture scores for the control and treated cheeses (Table 38) revealed that cheese made from psychrotrophic; coliform post-pasteurization contamination, high moisture and low activity starter had significantly (P < .001) lower body score than the control. Results shown in Table 57 indicate that *P. fluorescens* P27, coliform and high moisture cheese had significantly (P < .001) higher proteolytic activity than the control while Figures 67 and 68 show that cheese treated with *P. fluorescens* P27 had significantly (P < .05) higher short and long chain fatty acids than the control. Also, Figures 72 and 73 show both coliform treated and high moisture cheese had significantly (P < .05) higher linolenic acid (C18:3) than the control.

The predominant body/texture criticisms noted at 180 d in high moisture cheese were "weak" and "pasty." The criticisms "open" and "gassy" were noted in the cheese made from coliform contaminated pasteurized milk. At 60 d, "open" and "weak" criticisms were noted in cheese treated with *P. fluorescens* P27 (Tables 39 and 40).

As stated, all cheeses made with psychrotrophic contaminated raw milk resulted in cheese with significantly (P < .001) higher moisture content than the control (Table 16). This result supported data by Yan et al. (1983) who reported that moisture content increased for cheese made from raw milk held under prolonged
Table 38. Effect of psychrotrophic and coliform-
post-pasteurization contamination, high
moisture and low activity starter on
the body/texture of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Body/Texture Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>4.000 A*</td>
</tr>
<tr>
<td>2. P. fluorescens 27-10,000/ml</td>
<td>3.5833 B</td>
</tr>
<tr>
<td>3. P. fluorescens 27-100,000/ml</td>
<td>3.6500 B</td>
</tr>
<tr>
<td>4. Coliform</td>
<td>3.0500 C</td>
</tr>
<tr>
<td>5. High Moisture</td>
<td>3.1667 C</td>
</tr>
<tr>
<td>6. Low Activity</td>
<td>3.5625 B</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ signifi-
cantly (P < .001).

Table 39. Most frequently noted body/texture criticism
vs. time for control and treated cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1. Control</td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
</tr>
<tr>
<td>2. Psychrotroph</td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
</tr>
<tr>
<td>3. Coliform</td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
</tr>
<tr>
<td>4. High Moisture</td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
</tr>
<tr>
<td></td>
<td>Weak</td>
</tr>
</tbody>
</table>
Table 40. Frequency distribution of body criticisms of control and treated cheese at 180d.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Samples</th>
<th>Weak</th>
<th>Open</th>
<th>Curdy</th>
<th>Crumbly</th>
<th>Gassy</th>
<th>Pasty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>1(100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Psychrotroph</td>
<td>10</td>
<td>0</td>
<td>9(90 )</td>
<td>0</td>
<td>0</td>
<td>1(10 )</td>
<td>0</td>
</tr>
<tr>
<td>Coliform</td>
<td>5</td>
<td>0</td>
<td>5(50 )</td>
<td>0</td>
<td>0</td>
<td>5(50 )</td>
<td>0</td>
</tr>
<tr>
<td>High Moisture</td>
<td>5</td>
<td>5(50)</td>
<td>4(40 )</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(10 )</td>
</tr>
</tbody>
</table>

*Percent values based on total number of defects and not total number of samples. More than one criticism could be used for each sample.
Table 41. Microbiological profile of Cheddar cheese at different aging times.

<table>
<thead>
<tr>
<th>Days</th>
<th>SPC</th>
<th>Streptococci</th>
<th>Mean log</th>
<th>Coliform</th>
<th>CV</th>
<th>Lactobacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.2597 A</td>
<td>7.4918 A</td>
<td>2.4710 A</td>
<td>2.4927 A</td>
<td>8.3897 A</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.7907 B</td>
<td>6.5426 B</td>
<td>2.7576 A</td>
<td>2.2670 A</td>
<td>7.9112 B</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>7.8029 B</td>
<td>5.7933 C</td>
<td>2.2661 B</td>
<td>2.2110 A</td>
<td>7.8548 B</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>7.5315 B</td>
<td>5.6099 C</td>
<td>2.2214 B</td>
<td>1.8471 B</td>
<td>7.4163 C</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>6.5196 C</td>
<td>4.4299 D</td>
<td>1.0193 C</td>
<td>1.1186 C</td>
<td>6.3242 D</td>
<td></td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).
Figure 50. Microbiological profile of Cheddar cheese at different aging times.
Figure 51. Total bacterial count (SPC) of control vs. treated Cheddar cheese at different aging times.
Figure 52. Streptococcal population of control vs. treated Cheddar cheese at different aging times.
Figure 54. Psychrotrophic counts of control vs. treated Cheddar cheese at different aging times.
Figure 55. Coliform counts of control vs. treated Cheddar cheese at different aging times.
bulk starter with low bacterial activity exhibited significantly (P < .001) lower total bacterial count than the control and other treated cheeses (Figure 51).

**Effect of Psychrotrophic; Coliform Post-Pasteurization Contamination and High Moisture on Cheddar Cheese Yield**

Results shown in Table 42 indicate the actual cheese yield of high moisture cheese was significantly (P < .001) higher than the control and other treated cheeses. No significant differences were noted in the cheese yield between cheeses made from psychrotrophic and coliform post-pasteurization contaminated milk and the control cheese. The more realistic explanation for this situation is that the protease and lipase levels elaborated by psychrotrophic and coliform bacteria that were added directly to the vat were not high enough to cause excessive proteolysis and/or lipolysis respectively. This explanation is supported by Nelson and Marshall (1977) who reported 38% less curd was obtained in the presence of $10^9$/ml of *Enterobacter* spp.; also, yields decreased with an initial count of this bacterium of $2 \times 10^5$/ml and storage of 5°C for 48 h.

**Effect of Aging, Psychrotrophic and Coliform Post-Pasteurization Contamination, High Moisture and Low Activity Starter on the Proteolytic Activity of Cheddar Cheese**

Upon statistical examination of tyrosine content, the ANOV technique revealed significant (P < .001)
Table 42. Effect of psychrotrophic and coliform-post pasteurization contamination, high moisture and low activity starter on the yield of cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Yield-Weight (lb.)</th>
<th>Theoretical Yield (lb.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control Cheese</td>
<td>10.260 A&lt;sup&gt;1&lt;/sup&gt;</td>
<td>10.627 A&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>2. P. fluorescens 27-10,000/ml</td>
<td>10.228 A</td>
<td>10.970 A</td>
</tr>
<tr>
<td>3. P. fluorescens 27-100,000/ml</td>
<td>10.192 A</td>
<td>11.150 A</td>
</tr>
<tr>
<td>4. Coliform 1000/ml</td>
<td>10.192 A</td>
<td>11.074 A</td>
</tr>
<tr>
<td>5. High Moisture</td>
<td>11.288 B</td>
<td>11.696 B</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means not followed by the same letter differ significantly (P < .001).

<sup>2</sup>Means not followed by the same letter differ significantly (P < .01).
increases in tyrosine content between 0 and 180 d as shown in Table 43 and in Figures 56 and 57. This accounted for some increases in proteolytic activity with aging. The effect of psychrotrophs, coliforms and high moisture on proteolysis as measured by the Hull Test is demonstrated in Table 44 and in Figure 57, that the control cheese and cheese made from bulk starter with low bacterial activity had significantly ($P < .001$) less proteolysis over all days of storage than cheeses treated with psychrotrophs and coliform bacteria.

As stated previously (Table 14), the increase in tyrosine equivalent was always associated with an increase in the psychrotrophic count (Yan et al., 1983). This observation supported data by Gallman et al. (1982) who concluded that the contaminating flora of cheese (E. coli and P. fluorescens), which during processing of raw milk are normally characterized by the raw milk flora of the cheese milk, significantly influenced the cheese ripening, especially the proteolysis. Mainly concerned is the degradation of $\beta$-casein. Another alternative and more realistic explanation for high proteolytic activity in high moisture cheese, is that the more moisture in cheese, the more microbial (Figure 51) activity, enzymatic activity (Table 44) and more acidity (Table 52).
Table 43. Effect of aging on the proteolytic activity of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean Tyrosine Content (µg/5ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52.82 A*</td>
</tr>
<tr>
<td>5</td>
<td>70.41 A</td>
</tr>
<tr>
<td>30</td>
<td>130.81 B</td>
</tr>
<tr>
<td>60</td>
<td>196.21 C</td>
</tr>
<tr>
<td>180</td>
<td>332.34 D</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).

Table 44. Effect of psychrotroph and coliform post-pasteurization contamination, high moisture and low activity starter on the proteolytic activity of Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Tyrosine Content (µg/5ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control Cheese</td>
<td>135.14 A*</td>
</tr>
<tr>
<td>2. P. fluorescens 27-10,000/ml</td>
<td>167.05 B</td>
</tr>
<tr>
<td>3. P. fluorescens 27-100,000/ml</td>
<td>154.96 B</td>
</tr>
<tr>
<td>4. Coliform 1000/ml</td>
<td>159.99 B</td>
</tr>
<tr>
<td>5. High Moisture</td>
<td>219.47 C</td>
</tr>
<tr>
<td>6. Low Activity Starter</td>
<td>115.05 A</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
Figure 56. Effect of aging on the proteolytic activity of Cheddar cheese.
Figure 57. Proteolytic activity of control vs. treated Cheddar cheese at different aging times.
Effect of Aging on pH, Moisture and Salt of Cheddar Cheese

A significant (P < .001) increase in pH value was observed between 0 and 180 d as shown in Table 45. This increase in pH could be related to an increase in buffering capacity that resulted from increases in proteolytic activity in Cheddar cheese with aging at 7°C (Table 43).

As expected, data revealed in Table 47 demonstrate a significant (P < .001) drop in moisture content of Cheddar cheese between 0 and 180 d. This result was supported by Barraquio et al. (1982) who demonstrated that moisture content of Cheddar cheese was significantly affected by ripening period. Moisture content decreased while salt content increased (Table 46) significantly (P < .001) between 0 and 180 d. As stated, a decrease in moisture content was noted as the cheese ripened (Table 15) and this could be attributed to loss of moisture and the permeation of salt from the rind to the inner portion of the cheese as it ripens (Gomes, 1977).

Effect of Aging and Psychrotrophic and Coliform Post-Pasteurization Contamination, High Moisture and Low Activity Starter on Organic Acid Content and Flavor of Cheddar Cheese

Results of HPLC analysis shown in Tables 48, 49 and 50 and graphically in Figures 58 and 59 indicated significant (P < .001) increases of citric, pyruvic, lactic, formic, acetic and propionic acids with aging of the
### Table 45. Effect of aging on pH of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.1148 A*</td>
</tr>
<tr>
<td>5</td>
<td>5.1439 A</td>
</tr>
<tr>
<td>30</td>
<td>5.1313 B</td>
</tr>
<tr>
<td>60</td>
<td>5.1543 C</td>
</tr>
<tr>
<td>180</td>
<td>5.1709 C</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).

### Table 46. Effect of aging on salt content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean Salt Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.8809 A*</td>
</tr>
<tr>
<td>5</td>
<td>2.9117 B</td>
</tr>
<tr>
<td>30</td>
<td>2.9300 B</td>
</tr>
<tr>
<td>60</td>
<td>2.9522 B</td>
</tr>
<tr>
<td>180</td>
<td>3.0274 C</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
Table 47. Effect of aging on moisture content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean Moisture Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>38.45 A*</td>
</tr>
<tr>
<td>5</td>
<td>38.368 A</td>
</tr>
<tr>
<td>30</td>
<td>38.30 A</td>
</tr>
<tr>
<td>60</td>
<td>38.091 B</td>
</tr>
<tr>
<td>180</td>
<td>37.75 C</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
cheese. These results supported data by Irwin et al. (1984) who concluded that Cheddar cheese ripening showed a dramatic decrease in lactose during pressing with subsequent increases in lactic and citric acids. Further ripening yielded slight increases in other acids (pyruvic, acetic, propionic and butyric acids).

Results in Table 51 revealed a significantly (P < .001) lower level of citric acid value in control cheese than all other treated cheeses. Cheese made from coliform contaminated milk and bulk starter with low bacterial activity had significantly (P < .001) lower pyruvate values than the control and other treated cheeses. Both cheeses made from psychrotrophic post-pasteurization contaminated milk and high moisture curd revealed significantly (P < .001) higher lactic acid content than the control and other treated cheeses (Table 52). This observation was supported by Mikolajcik (1979) who concluded that the breakdown products produced by lipases and proteases elaborated by psychrotrophic bacteria, stimulate starter culture acid production which, in excess, produces body(texture and flavor defect. High moisture cheese (Table 52) had significantly (P < .001) more formic acid than the control and other treated cheeses. Results shown in Table 53 revealed that control cheese had significantly (P < .001) higher acetic acid value than all other treated cheeses. This observation supported data by Ohren and Tuckey
Table 48. Effect of aging on the citric and pyruvic acid content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Citric Mean ppm</th>
<th>Pyruvic Mean ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1543 A</td>
<td>33.66 A</td>
</tr>
<tr>
<td>5</td>
<td>1551 A</td>
<td>31.15 A</td>
</tr>
<tr>
<td>30</td>
<td>1676 B</td>
<td>43.59 B</td>
</tr>
<tr>
<td>60</td>
<td>1659 B</td>
<td>49.83 B</td>
</tr>
<tr>
<td>180</td>
<td>1688 B</td>
<td>66.84 C</td>
</tr>
</tbody>
</table>

* Means not followed by the same letter differ significantly (P < .01).

Table 49. Effect of aging on the lactic and formic acid content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Lactic Mean ppm</th>
<th>Formic Mean ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6909.6 A</td>
<td>175.85 A</td>
</tr>
<tr>
<td>5</td>
<td>7584.9 A</td>
<td>188.97 A</td>
</tr>
<tr>
<td>30</td>
<td>8240.8 B</td>
<td>223.26 B</td>
</tr>
<tr>
<td>60</td>
<td>8477.8 B</td>
<td>239.68 B</td>
</tr>
<tr>
<td>180</td>
<td>8784.4 B</td>
<td>287.07 C</td>
</tr>
</tbody>
</table>

* Means not followed by the same letter differ significantly (P < .01).
Table 50. Effect of aging on the acetic and propionic acid content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Acetic Mean ppm</th>
<th>Propionic Mean ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>110.74 A²</td>
<td>398.04 A</td>
</tr>
<tr>
<td>5</td>
<td>124.24 A</td>
<td>467.50 B</td>
</tr>
<tr>
<td>30</td>
<td>161.10 B</td>
<td>639.80 C</td>
</tr>
<tr>
<td>60</td>
<td>174.97 B</td>
<td>721.20 D</td>
</tr>
<tr>
<td>180</td>
<td>241.45 C</td>
<td>866.44 D</td>
</tr>
</tbody>
</table>

²Means in a column not followed by the same letter differ significantly (P < .001).
Figure 58. Effect of aging on the propionic, formic, acetic and pyruvic acid content of Cheddar cheese.
Figure 59. Effect of aging on the lactic and citric acid content of Cheddar cheese.
Table 51. Comparison of citric and pyruvic acids of control cheese with psychrotroph and coliform-post pasteurization contamination, high moisture and low activity starter cheese.

<table>
<thead>
<tr>
<th></th>
<th>Citric</th>
<th>Pyruvic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ppm</strong></td>
<td>-------</td>
<td>---------</td>
</tr>
<tr>
<td>1. Control</td>
<td>1472</td>
<td>61.14 A</td>
</tr>
<tr>
<td>2. <em>P. fluorescens</em> 27-10,000/ml</td>
<td>1645 B</td>
<td>52.10 A</td>
</tr>
<tr>
<td>3. <em>P. fluorescens</em> 27-100,000/ml</td>
<td>1745 B</td>
<td>53.93 A</td>
</tr>
<tr>
<td>4. Coliform 1000/ml</td>
<td>1588 B</td>
<td>36.78 B</td>
</tr>
<tr>
<td>5. High Moisture</td>
<td>1684 B</td>
<td>50.84 A</td>
</tr>
<tr>
<td>6. Low Activity</td>
<td>1568.3 B</td>
<td>22.41 B</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).
Table 52. Comparison of lactic and formic acids of control cheese with psychrotroph and coliform-post pasteurization contamination, high moisture and low activity starter cheese. (continued)

<table>
<thead>
<tr>
<th></th>
<th>Lactic</th>
<th>Formic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ppm</td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>6,415 A*</td>
<td>242.60 A</td>
</tr>
<tr>
<td>2. <em>P. fluorescens</em> 27-10,000/ml</td>
<td>9,117 B</td>
<td>236.77 A</td>
</tr>
<tr>
<td>3. <em>P. fluorescens</em> 27-100,000/ml</td>
<td>7,742 B</td>
<td>198.20 A</td>
</tr>
<tr>
<td>4. Coliform 1000/ml</td>
<td>6,397 A</td>
<td>209.87 A</td>
</tr>
<tr>
<td>5. High Moisture</td>
<td>10,403 C</td>
<td>327.3 B</td>
</tr>
<tr>
<td>6. Low Activity</td>
<td>6,360 A</td>
<td>166.97 A</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
(1969) who found that Cheddar cheese containing levels of acetate and free fatty acids, and whose acetate to fatty acid ratio was between specified limits, developed good flavor after 180 d ripening. Acetic acid has a less clear function since its concentration can vary considerably between cheeses without concomitant variability in the quality or intensity of typical flavor (Law and Castanon, 1976). It probably adds to the sharp mouthfeel of cheese conferred by the high lactic acid concentration, but overproduction of acetic acid can lead to vinegar-like off-flavors. Law (1982) concluded that mixtures of alkanolic acids with carbon chains from C₂ to C₈ or C₁₀ can impart cheese-like flavors either to naturally maturing cheese or in flavor mixtures for process cheese, but that their contribution to the aroma and the special character of Cheddar cheese is unproven.

Cheese made from bulk starter with low bacterial activity (Table 53) had significantly (P < .001) higher propionic acid values than the control and other treated cheeses. Marsili, Ostapenko, Simmons and Green (1981) reported that organic acids occur in dairy products as a result of the hydrolysis of butterfat (fatty acids) or bacterial growth (e.g., pyruvic, lactic, acetic and propionic acids). Also, they stated that quantitative determinations of these acids in dairy products is important to flavor studies, for nutritional reasons, and as indicator of bacterial activity.
Table 53. Comparison of acetic and propionic acids of control cheese with psychrotroph and coliform-post pasteurization contamination, high moisture and low activity starter cheese.
(continued)

<table>
<thead>
<tr>
<th></th>
<th>Acetic</th>
<th>Propionic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ppm</td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>224.5 A*</td>
<td>539.50 A</td>
</tr>
<tr>
<td>2. P. fluorescens</td>
<td>190.7 C</td>
<td>668.53 A</td>
</tr>
<tr>
<td>27-10,000/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. P. fluorescens</td>
<td>135.8 B</td>
<td>555.10 A</td>
</tr>
<tr>
<td>27-100,000/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Coliform</td>
<td>152.8 C</td>
<td>508.94 A</td>
</tr>
<tr>
<td>1000/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. High Moisture</td>
<td>242.2 A</td>
<td>627.82 A</td>
</tr>
<tr>
<td>6. Low Activity</td>
<td>80.4 B</td>
<td>850.00 B</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
Effect of Aging, Psychrotroph and Coliform Post-Pasteurization Contamination, High Moisture and Low Activity Starter on Volatile Organic Chemicals and Flavor of Cheddar Cheese

As stated in a previous section of this manuscript, other fat derived flavor compounds implicated in Cheddar flavor include ketones (Law, Castanon and Sharp, 1976). Figure 27 shows the chromatograms for a headspace analysis of aqueous volatile organic standards while Figures 28, 29, 30, 31 and 32 show the chromatograms for a headspace analysis of control Cheddar cheese samples at 0, 5, 30, 60 and 180 d, respectively. Figures 61 and 62 show the chromatograms for a headspace analysis of cheeses contaminated with coliform and low activity starter at 180 d, respectively.

Results of GCMS indicated in Tables 54 and 55 and graphically demonstrated in Figure 60 revealed that 60 and 180 d cheese had significantly (P < .001) higher acetone and 2-butanone while lower 2-pentanone values than the other days. Also, cheese at 0 d had significantly (P < .001) lower ethanol than cheese at the other days. The compound N-propanol peaked at 30 d followed by a significant (P < .001) drop at 60 and 180 d. Manning (1977) indicated that the 2-pentanone concentration in normal cheese is a good index of cheese age but the compound is not necessarily involved in flavor.

Gas chromatographic analysis with headspace sampling indicated that the control cheese had significantly
Figure 60. Effect of aging on ethanol, propanol, 2-pentanone, acetone and 2-butanone content of Cheddar cheese.
Figure 61. HSGC chromatograms of volatile organics in coliform-contaminated Cheddar cheese at 180 days of aging.
Figure 62. HSGC chromatograms of volatile organics in Cheddar cheese (treated with low activity starter) at 180 days of aging.
Table 54. Effect of aging on acetone, 2-butanolone and ethanol content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Acetone</th>
<th>2-Butanone</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0261 A</td>
<td>0.5213 A</td>
<td>62.823 A</td>
</tr>
<tr>
<td>5</td>
<td>1.0283 A</td>
<td>0.5330 A</td>
<td>64.388 A</td>
</tr>
<tr>
<td>30</td>
<td>1.0913 A</td>
<td>0.5404 A</td>
<td>70.298 B</td>
</tr>
<tr>
<td>60</td>
<td>1.1143 B</td>
<td>0.5456 A</td>
<td>71.475 B</td>
</tr>
<tr>
<td>180</td>
<td>1.1483 B</td>
<td>0.5808 B</td>
<td>67.206 A</td>
</tr>
</tbody>
</table>

1 Means not followed by the same letter differ significantly ($P < .001$).

Table 55. Effect of aging on 2-pentanone and N-propanol content of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>2-Pentanone</th>
<th>N-Propanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.2263 A</td>
<td>1.5741 A</td>
</tr>
<tr>
<td>5</td>
<td>1.3194 A</td>
<td>1.5959 A</td>
</tr>
<tr>
<td>30</td>
<td>1.2600 A</td>
<td>1.7147 B</td>
</tr>
<tr>
<td>60</td>
<td>1.2217 A</td>
<td>1.6347 C</td>
</tr>
<tr>
<td>180</td>
<td>1.0679 B</td>
<td>1.6100 C</td>
</tr>
</tbody>
</table>

1 Means not followed by the same letter differ significantly ($P < .001$).

2 Means not followed by the same letter differ significantly ($P < .05$).
(P < .001) lower acetone and 2-butanone values than all the other treatments (Table 56). Upon statistical examination of organic volatile chemicals, the ANOV technique revealed significant (P < .001) increases of propanol (Figure 61) and decreases of 2-pentanone due to contamination with coliform as shown in Table 57. Both high moisture and psychrotrophic contaminated cheeses had significantly (P < .001) higher ethanol values than the control (Table 57), while cheese made from bulk starter with low bacterial activity compared to all control and other treated cheeses had significantly lower concentration of ethanol (Figure 62 and Table 57). Keen and Walker (1974) observed wide variations in the concentrations of 2-butanone even in Cheddar cheeses made using the same single-strain starter (Streptococcus cremoris AM₂). Our results supported data by Scarpellino (1961) who concluded that 2-butanone contributes to the desirable flavor complex of Cheddar cheese whereas the reduction product 2-butanol is associated with off-flavor development.

The lower mean flavor score demonstrated by the coliform contaminated Cheddar cheese could account for high concentration of propanol and 2-butanone but lower concentration of 2-pentanone. High moisture and psychrotrophic contaminated cheese had significantly (P < .001) lower flavor score (Table 34) due to high levels of
Table 56. Comparison of control cheese with psychrotrophic and coliform-post pasteurization contamination, high moisture and low activity starter cheese with regard to acetone and 2-butanone.

<table>
<thead>
<tr>
<th></th>
<th>Acetone</th>
<th>2-Butanone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Control</strong></td>
<td>0.8380 A</td>
<td>0.4346 A</td>
</tr>
<tr>
<td><strong>2. P. fluorescens</strong>&lt;br&gt;27-10,000/ml</td>
<td>1.1500 B</td>
<td>0.5560 B</td>
</tr>
<tr>
<td><strong>3. P. fluorescens</strong>&lt;br&gt;27-100,000/ml</td>
<td>1.1544 B</td>
<td>0.5524 B</td>
</tr>
<tr>
<td><strong>4. Coliform</strong>&lt;br&gt;1000/ml</td>
<td>1.0608 B</td>
<td>0.5572 B</td>
</tr>
<tr>
<td><strong>5. High Moisture</strong></td>
<td>1.1673 B</td>
<td>0.5626 B</td>
</tr>
<tr>
<td><strong>6. Low Activity</strong></td>
<td>1.0840 B</td>
<td>0.5775 B</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).*
Table 57. Comparison of control cheese with psychrotrophic and coliform-post pasteurization contamination, high moisture and low activity starter cheese with regard to ethanol, 2-pentanone and propanol.

<table>
<thead>
<tr>
<th></th>
<th>Ethanol</th>
<th>2-Pentanone</th>
<th>Propanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Control</strong></td>
<td>78.72 A</td>
<td>1.633 A</td>
<td>0.864 A</td>
</tr>
<tr>
<td><strong>2. P. fluorescens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-10,000/ml</td>
<td>103.78 B</td>
<td>1.31 B</td>
<td>0.866 A</td>
</tr>
<tr>
<td><strong>3. P. fluorescens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-100,000/ml</td>
<td>70.43 A</td>
<td>1.26 B</td>
<td>0.832 A</td>
</tr>
<tr>
<td><strong>4. Coliform</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000/ml</td>
<td>58.83 A</td>
<td>0.16 C</td>
<td>3.628 B</td>
</tr>
<tr>
<td><strong>5. High Moisture</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.12 B</td>
<td>1.36 A</td>
<td>0.893 A</td>
</tr>
<tr>
<td><strong>6. Low Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.59 C</td>
<td>1.31 B</td>
<td>0.824 A</td>
</tr>
</tbody>
</table>

1Means in a column not followed by the same letter differ significantly (P < .001).
ethanol, acetone and 2-butanone. These results were consistent with Marsili (1981) who reported that high levels of alcohol and acetone impaired cheese quality. The predominant flavor criticism in high moisture cheese (Table 35) was fermented fruity and this could be attributed to its high ethanol concentration. This occurrence supported data by others (McGugan et al., 1975; Manning, 1979) who reported that the fruity defect occurs in cheeses which contain high ethanol level.

Although cheese treated with psychrotrophs did not result in fruity flavor, it had significantly (P < .001) higher ethanol values than the control. Law (1982) suggested that the fruity defect can be caused by high ethanol levels produced by psychrotrophic bacteria.

**Chemical Profiles of "Good" Versus "Bad" Cheddar Cheese**

Cheddar cheese from each vat was subjected to sensory evaluation by a four-member trained panel at 5, 30, 60 and 180 d. Modified ADSA Cheddar cheese scorecard was used to score the flavor (1-10 scale). Those samples having a flavor score of ≥7 were considered "good" while those having a flavor score <7 were considered "fair to bad." Mean chemical profiles of these two groups were then compared to note similarities and differences in composition. The control cheese had significantly (P < .01) higher flavor scores than cheese made from
psychrotrophic or coliform post-pasteurization contaminated milk, high moisture and low activity starter (Table 34). Statistically (P < .001) higher levels of citrate, acetone, 2-butanone, proteolytic activity, lactate, ethanol, propanol, formate and propionate, but lower levels of acetate, 2-pentanone, pyruvate, pH and salt were found in "bad" Cheddar cheese as opposed to "good" Cheddar cheese (Tables 58, 59, 60 and 61).

Mikolajcik (1979) concluded that the breakdown products produced by lipases and proteases elaborated by psychrotrophic bacteria, stimulate starter culture acid production, which, in excess, produces body/texture and flavor defect. Ohren and Tuckey (1969) found Cheddar cheese whose acetate to fatty acid ratio was between specified limits developed good flavor after 180 d ripening.

Statistically higher levels of butyrate (P < .05) (Table 64), caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic (P < .001) (Tables 65 and 66) and linolenic acid (P < .05) (Table 67) were observed in "bad" Cheddar cheese as opposed to "good" Cheddar cheese.

Generally, free fatty acids have been acknowledged to contribute cheesiness in Cheddar cheese (Forss, 1979). However, claims have been made that fatty acids are important because low fat or fat free cheeses did not
Table 58. Comparison of cheese made from post-pasteurization contaminated psychrotroph milk and control cheese.

<table>
<thead>
<tr>
<th>Higher than Control</th>
<th>Lower than Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (P &lt; .001)</td>
<td>Sig. (P &lt; .001)</td>
</tr>
<tr>
<td>Citrate</td>
<td>Acetate</td>
</tr>
<tr>
<td>Acetone</td>
<td>2-Pentanone</td>
</tr>
<tr>
<td>2-Butanone</td>
<td></td>
</tr>
<tr>
<td>Proteolytic</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
</tr>
</tbody>
</table>

Table 59. Comparison of cheese made from coliform-contaminated milk and control cheese.

<table>
<thead>
<tr>
<th>Higher than Control</th>
<th>Lower than Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (P &lt; .001)</td>
<td>Sig. (P &lt; .001)</td>
</tr>
<tr>
<td>Citrate</td>
<td>Acetate</td>
</tr>
<tr>
<td>Acetone</td>
<td>2-Pentanone</td>
</tr>
<tr>
<td>2-Butanone</td>
<td></td>
</tr>
<tr>
<td>Proteolytic</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
</tr>
<tr>
<td>Propanol</td>
<td></td>
</tr>
</tbody>
</table>
Table 60. Comparison of high moisture and control cheeses.

<table>
<thead>
<tr>
<th>Higher than Control</th>
<th>Lower than Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. ( (P &lt; .001) )</td>
<td>Sig. ( (P &lt; .001) )</td>
</tr>
<tr>
<td>Citrate</td>
<td>pH</td>
</tr>
<tr>
<td>Acetone</td>
<td>Salt</td>
</tr>
<tr>
<td>2-Butanone</td>
<td></td>
</tr>
<tr>
<td>Proteolytic</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td>Formate</td>
<td></td>
</tr>
</tbody>
</table>

Table 61. Comparison of cheese made from bulk starter with low bacterial number and control cheese.

<table>
<thead>
<tr>
<th>Higher than Control</th>
<th>Lower than Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. ( (P &lt; .001) )</td>
<td>Sig. ( (P &lt; .001) )</td>
</tr>
<tr>
<td>Citrate</td>
<td>Acetate</td>
</tr>
<tr>
<td>Acetone</td>
<td>2-Pentanone</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>Pyruvate</td>
</tr>
<tr>
<td>Propionate</td>
<td>Ethanol</td>
</tr>
<tr>
<td></td>
<td>Proteolytic</td>
</tr>
</tbody>
</table>
develop flavor (Ohren and Tuckey, 1969). Woo et al. (1984) concluded that aged, desirably-flavored Cheddar cheese has intermediate concentrations of individual FFA. Also, he concluded that some excellent Cheddar cheese has low concentrations of the short chain FFA. When conditions permit development of more FFA in Cheddar cheese, rancid off-flavors are readily apparent. This is specifically true for butyric acid (Woo et al., 1984). Sharp (1972) concluded that psychrotrophic lipases gave rise to rancid-flavored Cheddar cheeses containing high levels of free butyric acid and higher fatty fatty acids.

It seems to be as Marsili stated (1981) that the aroma and flavor of high quality Cheddar cheese are attributed to a delicate balance of organic acids and volatile organic chemicals produced as metabolites by culture bacteria during fermentation. Mulder (1952) proposed what is now known as the Component Balance Theory (CBT). This theory suggested that Cheddar flavor was made up of a balance of flavors contributed by a number of different compounds. When the balance was upset by an excess or lack of one or more of the component compounds, atypical flavor was produced.

Effect of Aging, Psychrotrophs and Coliform Post-Pasteurization Contamination, High Moisture and Low Activity Starter on Free Fatty Acid Content and Flavor of Cheddar Cheese

Generally, free fatty acids have been acknowledged to contribute cheesiness in Cheddar flavor (Forss, 1979).
However, claims that fatty acids are important because low-fat or fat-free cheeses do not develop flavor (Ohren and Tuckey, 1969) are oversimplistic since deviations from an optimum fat percentage in the product alters its characteristics markedly and may have indirect effects on flavor retention and perception. It has been documented that skim milk cheese has considerably lower levels of free fatty acids than normal cheese (Deane, 1972; Deane and Dolan, 1973; Foda et al., 1974; Dulley and Grieve, 1974).

Data shown in Tables 62 and 63 of gas chromatographic analysis over all days of storage indicated significant (to at least < .05) increases of caproic, caprylic, lauric, myristic, palmitic, stearic and oleic with aging of the cheese (Figures 63, 64, 65 and 66). These results support data in the first section of this manuscript. Although changes in concentrations of capric and linoleic acids during aging were not statistically significant (P > .05) (Tables 62 and 63), they increased with aging as shown in Figure 64. These results supported data by Woo et al. (1984) who concluded that young Cheddar cheese contains low free fatty acids, and aged desirably-flavored Cheddar cheese has intermediate concentrations of individual FFA. Stadhouders and Veringa (1973) also reported that the amount of volatile free fatty acids increases during Cheddar cheese maturation.
Table 62. Effect of aging on free fatty acid content (C6-C14) of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Caproic (C6)</th>
<th>Caprylic (C8)</th>
<th>Capric (C10)</th>
<th>Lauric (C12)</th>
<th>Myristic (C14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>5</td>
<td>16.013 A</td>
<td>11.898 A</td>
<td>28.012 A</td>
<td>36.504 A</td>
<td>114.71 A</td>
</tr>
<tr>
<td>30</td>
<td>17.575 A</td>
<td>12.810 AB</td>
<td>30.397 AB</td>
<td>40.577 AB</td>
<td>128.04 AB</td>
</tr>
<tr>
<td>60</td>
<td>15.579 A</td>
<td>11.606 A</td>
<td>27.544 A</td>
<td>34.932 A</td>
<td>119.02 A</td>
</tr>
<tr>
<td>180</td>
<td>20.337 A</td>
<td>15.017 B</td>
<td>33.349 B</td>
<td>44.147 B</td>
<td>148.39 B</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .05).
Table 63. Effect of aging on free fatty acid content (C16-C18:2) of Cheddar cheese.

<table>
<thead>
<tr>
<th>Days</th>
<th>Palmitic (C16)</th>
<th>Stearic (C18)</th>
<th>Oleic (C18:1)</th>
<th>Linoleic (C18:2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>377.75 A*</td>
<td>219.64 A**</td>
<td>257.47 A</td>
<td>41.699 AB</td>
</tr>
<tr>
<td>5</td>
<td>381.34 A</td>
<td>222.31 A</td>
<td>254.65 A</td>
<td>39.971 AB</td>
</tr>
<tr>
<td>30</td>
<td>424.78 AB</td>
<td>261.43 AB</td>
<td>280.05 AB</td>
<td>42.454 AB</td>
</tr>
<tr>
<td>60</td>
<td>395.20 A</td>
<td>249.00 A</td>
<td>249.39 A</td>
<td>37.089 A</td>
</tr>
<tr>
<td>180</td>
<td>477.79 B</td>
<td>303.25 B</td>
<td>307.13 B</td>
<td>46.502 B</td>
</tr>
</tbody>
</table>

* Means in a column not followed by the same letter differ significantly (P < .05).
** Means in a column not followed by the same letter differ significantly (P < .01).
Figure 63. Effect of aging on free fatty acid content (C6, C8 and C18:3) of Cheddar cheese.
Figure 64. Effect of aging on free fatty acid content (C4, C10, C12, C18:2) of Cheddar cheese.
Figure 65. Effect of aging on free fatty acid content (C14) of Cheddar cheese.
Figure 66. Effect of aging on free fatty acid content (C16, C18, C18:1) of Cheddar cheese.
Upon statistical examination of butyric (C4) and linolenic (C18:3) acids, the ANOV technique revealed significant (P < .008 for C4, P < .0001 for C18:3) interaction between days of storage and treatments. This means at different levels of treatments, a different pattern was observed over time. In this case, days cannot be ignored within treatments and treatments cannot be ignored within days. For that reason, TD (Treatment-Day) analysis was used (Tables 64 and 67).

Mean values for butyric acid (Table 64) revealed that both high moisture and psychrotrophic contaminated cheese had significantly (P < .05) higher butyrate values than the control (Figure 67).

The predominant flavor criticism in cheese made from psychrotrophic post-pasteurization contaminated milk was rancid (Table 35) and this could be related to the higher butyric acid concentration (Table 64). This result supported data by Woo et al. (1984) who concluded that when conditions permit development of more FFA in Cheddar, rancid off-flavors are readily apparent. This is specifically true for butyric acid. Data shown in Tables 65 and 66 indicated that control cheese had significantly (P < .001) lower caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic and linoleic than cheese made from psychrotrophic (P. fluorescens P27 100,000/ml) post-pasteurization contaminated milk. Data
shown in Figure 68 indicated that control cheese had significantly (P < .05) lower capric, lauric, palmitic, stearic, oleic and linolenic at 180 d of aging than cheese made from psychrotrophic (P27) contaminated milk. These observations supported data of the previous section of this manuscript (Table 26). In addition, previous investigators (Reiter, Fryer and Sharp, 1965; Sharp, 1972) concluded that psychrotrophic lipases gave rise to rancid flavored Cheddar cheeses containing high levels of free butyric acid and higher fatty acids. Christen et al. (1980 and 1984) concluded that the highest incidence of lipolytic activity among the psychrotrophic gram negative flora of commercial raw milk was found in *Pseudomonas fluorescens* 27 (P27).

In addition, by 60 d significantly (P < .05) higher linolenic acid concentration was noted in Cheddar cheese made from milk contaminated with psychrotrophs and coliforms than the control cheese (Table 67). As noted in Figure 69, 180 d cheese had significantly (P < .001) higher linolenic acid value than 30 d cheese for those made from milk contaminated with psychrotroph.

Data revealed in Figure 67 demonstrate significantly (to at least P < .05) higher volatile fatty acids (C6 and C8) in cheese contaminated with psychrotrophs than the control cheese. Patton (1963) claimed that volatile fatty acids (C2-C8) were the "backbone" of Cheddar aroma
Table 64. Comparison of butyric acid of control cheese with psychrotrophic and coliform post-pasteurization contaminated, high moisture and low activity starter.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>180</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Control</td>
<td>18.42</td>
<td>30.12</td>
<td>22.31</td>
<td>32.96</td>
<td>39.20</td>
<td>28.62</td>
</tr>
</tbody>
</table>
| *Means in a column not followed by the same letter differ significantly (P < .05).*

(P. fluorescens P27 10,000/ml) 27.98 A 23.59 A 47.18 B 58.97 B 36.73 ABC 38.89

(P. fluorescens P27 100,000/ml) 41.89 AB 55.21 B 36.73 AB 25.46 C 58.74 A 43.61

Coliform 1000/ml 28.52 A 22.87 A 22.84 A 42.17 ABC 33.50 BC 29.48

High Moisture 47.23 B 40.96 AB 37.91 AB 62.59 B 36.17 ABC 44.97

Low Bacterial Activity 21.31 A 26.67 A 44.59 AB 18.67 C 50.29 AB 32.31
**Table 65.** Comparison of free fatty acids (C6-C12) of control cheese with psychrotrophic and coliform post-pasteurization contaminated, high moisture and low activity starter cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Caproic C6</th>
<th>Caprylic C8</th>
<th>Capric C10</th>
<th>Lauric C12</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens P27 10,000/ml</em></td>
<td>18.77 A</td>
<td>13.35 AB</td>
<td>30.98 AB</td>
<td>39.55 AB</td>
</tr>
<tr>
<td><em>P. fluorescens P27 100,000/ml</em></td>
<td>25.36 B</td>
<td>16.60 B</td>
<td>38.50 B</td>
<td>52.58 B</td>
</tr>
<tr>
<td>Coliform 1000/ml</td>
<td>14.37 A</td>
<td>11.35 A</td>
<td>27.50 A</td>
<td>35.04 A</td>
</tr>
<tr>
<td>High Moisture</td>
<td>16.38 A</td>
<td>11.20 A</td>
<td>24.36 A</td>
<td>31.51 A</td>
</tr>
<tr>
<td>Low Bacterial Activity</td>
<td>16.89 A</td>
<td>13.48 AB</td>
<td>31.67 AB</td>
<td>42.43 AB</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .001).*
Table 66. Comparison of free fatty acids (C14-C18:2) of control cheese with psychrotrophic and coliform post-pasteurization contaminated, high moisture and low activity starter cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Myristic C14</th>
<th>Palmitic C16</th>
<th>Stearic C18</th>
<th>Oleic C18:1</th>
<th>Linoleic C18:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>104.11 A*</td>
<td>336.95 A*</td>
<td>200.84 A*</td>
<td>231.76 A*</td>
<td>38.85 A*</td>
</tr>
<tr>
<td><em>P. fluorescens</em> P27 10,000/ml</td>
<td>135.65 AB</td>
<td>468.8 AB</td>
<td>318.84 BC</td>
<td>281.41 A</td>
<td>43.16 A</td>
</tr>
<tr>
<td><em>P. fluorescens</em> P27 100,000/ml</td>
<td>168.82 B</td>
<td>558.64 B</td>
<td>362.01 C</td>
<td>405.55 B</td>
<td>59.11 B</td>
</tr>
<tr>
<td>Coliform 1000/ml</td>
<td>113.62 A</td>
<td>359.49 A</td>
<td>191.12 A</td>
<td>259.45 A</td>
<td>39.21 A</td>
</tr>
<tr>
<td>High Moisture</td>
<td>100.71 A</td>
<td>353.30 A</td>
<td>239.27 AB</td>
<td>197.32 A</td>
<td>30.90 A</td>
</tr>
<tr>
<td>Low Bacterial Activity</td>
<td>125.58 A</td>
<td>391.66 A</td>
<td>194.66 A</td>
<td>242.93 A</td>
<td>38.03 A</td>
</tr>
</tbody>
</table>

*Means not followed by the same letter differ significantly (P < .001).
Table 67. Comparison of linolenic acid of control cheese with psychrotrophic and form post-pasteurization contaminated, high moisture and low activity starter cheese.

<table>
<thead>
<tr>
<th></th>
<th>Days</th>
<th>0</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. fluorescens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P27 10,000/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 A</td>
<td>0 A*</td>
<td>0 A*</td>
<td>0 A*</td>
<td>16.07 A*</td>
<td></td>
</tr>
<tr>
<td>P. fluorescens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P27 100,000/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 A</td>
<td>7.94 B</td>
<td>24.13 B</td>
<td>18.22 B</td>
<td>27.61 B</td>
<td></td>
</tr>
<tr>
<td>Coliform</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 A</td>
<td>0 A</td>
<td>18.76 C</td>
<td>17.83 B</td>
<td>17.76 A</td>
<td></td>
</tr>
<tr>
<td>High Moisture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 C</td>
</tr>
<tr>
<td>Low Bacterial Activity</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 A</td>
<td>0 C</td>
</tr>
</tbody>
</table>

*Means in a column not followed by the same letter differ significantly (P < .05).
Figure 67. Free fatty acid profile of psychrotrophic-post pasteurization contaminated Cheddar cheese versus control at 30 days aging.
Figure 68. Free fatty acid profile of psychrotroph-post pasteurization contaminated Cheddar cheese versus control at 180 days of aging.
Figure 69. Free fatty acid profile of psychrotrophic-post pasteurization contaminated Cheddar cheese at 30 days versus 180 days.
while Manning and Price (1977) claimed that these acids were only important in the background taste of the cheese. Also, Kosikowski and Iwasaki (1975); Sood and Kosikowski (1979) and Woo et al. (1984) suggested that increased free volatile fatty acids (short-chain FFA) in lipase-treated American Cheddar increase its flavor intensity, provided that high rancidity-inducing lipase levels are avoided. In addition, Woo et al. (1982) concluded that some apparently fine-flavored Cheddar cheeses have low concentration of the short-chain FFA.

Data revealed in Figures 70 and 71 demonstrate a significant (P < .05) difference in capric and lauric at 30 d and caprylic and linolenic at 180 d between cheese made from bulk starter with bacterial activity versus the control cheese. By 30 d the control cheese had significantly (P < .05) lower capric and lauric acid values. By 180 d the control cheese had significantly (P < .05) lower caprylic acid value and higher linolenic acid value than cheese made from bulk starter with low bacterial activity.

Data revealed in Figure 72 demonstrated that by 180 d the control cheese had significantly (P < .05) higher linolenic acid value than the high moisture Cheddar cheese. By 30 d the control cheese had significantly (P < .05) lower linolenic acid value than cheese contaminated with coliform (Figure 73). In addition,
high moisture Cheddar cheese had significantly ($P < .05$) higher stearic acid value at 180 than at 30 d (Figure 74). Finally, data agree with the conclusion of Cogan (1980) when he reported that the deteriorative effect of heat-resistant microbial lipases on cheese quality was due to psychrotrophic bacteria. Also, it was found that *Pseudomonas fluorescens* 27 (P27) demonstrated phospholipases activity on lecithin agar where it produced clean zones around the colonies. These zones were attributed to activity of phospholipase A$_1$ or A$_2$ due to the formation of water-soluble lysolecithin (Chrisope et al., 1976).

In general, fat plays a very important role in the development of a good texture, and it is well known that a higher fat content leads to a less firm and elastic body. These differences can be explained by the presence of more protein matrix in the cheese (Emmons et al., 1980).

The normal levels of fatty acids found in cheese (approximately 500 ppm) represent amounts well above typical flavor and aroma thresholds, ranging from 0.3-100 ppm (Baldwin et al., 1973). Thus, the fatty acids would be expected to contribute to the overall organoleptic qualities of Cheddar cheese.

Bynum et al. (1984) reported that large increases of total free fatty acids in a Cheddar cheese sample correlated with development of rancid flavors. Moreover,
Figure 70. Free fatty acid profile (C10-C12) of Cheddar cheese made from bulk starter with low bacterial activity versus control at 30 days of aging.
Figure 71. Free fatty acid profile (C8, C18:3) of Cheddar cheese made from bulk starter with low bacterial activity versus control at 180 days of aging.
Figure 72. Free fatty acid profile (C18:3) of high moisture Cheddar cheese versus control at 180 days of aging.
Figure 73. Free fatty acid profile (C18:3) of coliform contaminated Cheddar cheese versus control at 30 days of aging.
Free fatty acid profile (C18) of high moisture Cheddar cheese at 30 days versus 180 days of aging.
phospholipids in the cheeses decreased by 50-70% during the 6 mo ripening. The breakdown of phospholipids during ripening of Cheddar cheeses was attributed to phospholipases produced by psychrotrophic bacteria (Chrisope and Marshall, 1976).

Prediction of Cheese Flavor

Using stepwise regression in "Part I," and the variables on 0, 5 and 30 d, regression equations were formulated to predict the flavor of Cheddar cheese on 180 d. Data in "Part II" of this research were utilized in the same manner in order to test the repeatability of equations established in "Part I." Although variables in the regression equations of "Part II" differed from "Part I," certain similarities did exist which may be beneficial in flavor prediction.

When stepwise regression was used in "Part II" without inclusion of free fatty acids as shown (Table 68), the substitution of coliform and salt at 5 d, streptococci count at 30 d, propionic acid value at 0 d and acetone at 30 d (Table 68), revealed a regression equation with a coefficient of determination \( R^2 \) of .79. This meant that 79% of the variation in flavor could be explained by changes in these variables. With inclusion of free fatty acids to all other variables, the substitution of acetic acid at 5 d, lactic and palmitic acid at 30 d as shown (Table 69), revealed a regression
Table 68. Regression coefficients for equation\(^2\) to predict ripened Cheddar cheese flavor\(^1\) by analysis of young cheese.

<table>
<thead>
<tr>
<th>Variable</th>
<th>b Value</th>
<th>Prob.&gt;F</th>
<th>(R^2)</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.597</td>
<td></td>
<td></td>
<td>6.39</td>
</tr>
<tr>
<td>X1 Coliform (day 5)</td>
<td>-.3031</td>
<td>.0082</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>X2 Salt (day 5)</td>
<td>2.2671</td>
<td>.0033</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3 Streptococci (day 30)</td>
<td>0.3908</td>
<td>.0003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X4 Propionic (day 0)</td>
<td>-.0043</td>
<td>.0023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X5 Acetone (day 30)</td>
<td>-2.3213</td>
<td>.0221</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Flavor at d 180.

\(^2\)Regression equation calculated as follows:
\[ Y = (2.597) + (-.3031)(X1) + (2.267)(X2) + (0.3908)(X3) + (-.0043)(X4) + (-2.3213)(X5) \]
Table 69. Regression coefficients for equation to predict ripened Cheddar cheese flavor by analysis of young cheese.

<table>
<thead>
<tr>
<th>Variable</th>
<th>b Value</th>
<th>Prob.&gt;F</th>
<th>R²</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>6.477</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X1 Acetic Acid (day 5)</td>
<td>0.0211</td>
<td>0.0001</td>
<td>0.94</td>
<td>7.10</td>
</tr>
<tr>
<td>X2 Lactic Acid (day 30)</td>
<td>-0.0001</td>
<td>0.0019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3 Palmitic Acid (day 30)</td>
<td>-0.0016</td>
<td>0.0163</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Flavor at d 180.

2 Regression equation calculated as follows:
\[ Y = (6.477)+(0.0211)(X1)+(-0.0001)(X2)+(-0.0016)(X3) \]
Table 70. Regression coefficients for equation$^2$ to predict ripened Cheddar cheese flavor$^1$ by analysis of young cheese including additional variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>b Value</th>
<th>Prob.&gt;F</th>
<th>$R^2$</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>14.433</td>
<td></td>
<td>0.996</td>
<td></td>
</tr>
<tr>
<td>X1 pH (day 0)</td>
<td>-2.3502</td>
<td>0.041</td>
<td>7.10</td>
<td></td>
</tr>
<tr>
<td>X2 Salt (day 30)</td>
<td>1.3065</td>
<td>0.019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3 Lactic Acid (day 5)</td>
<td>0.0002</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X4 Acetic Acid (day 5)</td>
<td>0.0254</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X5 Lactic Acid (day 30)</td>
<td>-0.0002</td>
<td>0.0010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X6 Hull (day 30)</td>
<td>-0.0173</td>
<td>0.0011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X7 Lauric Acid (day 30)</td>
<td>0.0131</td>
<td>0.129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X8 Palmitic Acid (day 30)</td>
<td>-0.0025</td>
<td>0.017</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$Flavor at d 180.

$^2$Regression equation calculated as follows:

$$Y = (14.433) + (-2.350)(X1) + (1.306)(X2) + (0.0002)(X3) + (0.025)(X4) + (-0.0002)(X5) + (-0.0173)(X6) + (0.0131)(X7) + (-0.0025)(X8)$$
equation with a coefficient of determination ($R^2$) of .94. This meant that 94% of the variation in flavor could be explained by changes in these variables. With the addition of more variables as shown (Table 70), the $R^2$ could be increased to .996. The determination of this many variables would be unwieldy for most dairy plant laboratories. In addition, significant correlations of acetic acid to flavor were observed on 0 d (Table 31) and 30 d (Table 33) in "Part I" of this research and on 5 d (Tables 69 and 70) in "Part II". This indicates that acetic acid may be involved directly or serves as a good indicator of Cheddar cheese flavor.

Also, significant correlations of pH to flavor were observed on 5 d (Table 31), 0 d (Tables 32 and 33) in "Part I" of this research and on 0 d (Table 70) in "Part II". Likewise, this indicates that pH may be involved directly or serves as a good indicator of Cheddar cheese flavor. Also observed was a significant negative correlation of proteolytic activity at 5 d as measured by the Hull Test (Table 33) in "Part I" and at 30 d (Table 70) in "Part II" indicating that the higher the proteolytic activity in young cheese, the lower the flavor score at 180 d.

The moisture content which accounted for much of the variation in flavor in "Part I" (Tables 29 and 31) and in "Part II" (Table 34), did not appear in the regression
equations of "Part II" (Tables 68, 69 and 70). Even so, significant correlation of the moisture content to Cheddar cheese flavor was observed on 0 d (Tables 29 and 31). Thus, it was concluded that the moisture content of cheese may still be useful in flavor prediction.

The data in "Parts I and II" of this research did not give an absolute prediction of flavor; however, data were useful in providing avenues for additional study. Further testing of these equations (Tables 31, 32, 33, 68, 69 and 70) on actual samples would be necessary before definite conclusions could be drawn.
CHAPTER V
SUMMARY AND CONCLUSIONS

The main objective of this research was to determine what effect psychrotrophic contamination of raw and pasteurized milk, post-pasteurization contamination with coliforms, high moisture curd and low activity starter would have on major chemical components, yield and flavor of Cheddar cheese. It was also of interest to establish chemical profiles of "good" versus "bad" Cheddar cheese and to gain information which would aid in the prediction of ripened Cheddar cheese flavor by analysis of young cheese.

Under conditions of this study, the significant \( P < .001 \) highest mean flavor and body/texture scores over all days of storage were observed in control cheese. In addition, the significant \( P < .001 \) lowest mean flavor and body/texture scores were observed in all other treated cheeses. The predominant flavor criticisms at 180 d of cheese treated with psychrotrophs, coliforms and high moisture curd were bitter and unclean. Also, high moisture cheese exhibited fermented and whey taint in addition to the bitter and unclean flavors. The predominant body/texture criticisms noted at 60 and 180 d in psychrotrophic treated cheese were weak and open while
in high moisture cheese the criticisms were weak and pasty. In coliform-treated cheese, the predominant criticisms were open and gassy. All cheeses made with psychrotrophic contaminated raw milk resulted in cheese with significantly (P < .001) higher moisture content than the control.

Cheddar cheese yield decreased as counts of psychrotrophs in raw milk decreased during storage. This could be related with increases in proteolysis of the raw milk. High moisture cheese had significantly (P < .001) higher cheese yield than the control and other treated cheeses. A significant (P < .001) drop in moisture content and increases in pH and salt content with aging of cheese were observed.

Results of HPLC analysis indicated significant (P < .001) increases of citric, pyruvic, lactic, formic, acetic and propionic acids with aging; only orotic acid decreased with aging. Results from gas chromatographic analysis of free fatty acids (FFA) indicated significant (to at least .05) increases of butyric, caproic, caprylic, capric, lauric, myristic, palmitic and oleic acids with aging of the cheese. Ketones (fat-derived flavor compounds) were also implicated in Cheddar flavor. Results indicated that 60 and 180 d cheese had significantly (P < .001) higher acetone and 2-butanone values and lower 2-pentanone values than cheese at the other days.
Young Cheddar cheese contained low concentrations of individual free fatty acids and aged desirably-flavored Cheddar cheese had intermediate concentrations of individual FFA.

All the microbiological counts showed that lactobacilli, streptococci and total aerobic counts tended to decrease with days of storage and were significantly (P < .001) lower at both 60 and 180 d. At 0 d both psychrotrophic and coliform counts were significantly (P < .001) higher than the other days.

Mean chemical profiles of "good" versus "bad" Cheddar cheese over all days of storage were compared to note similarities and differences in composition. Statistically significant (P < .001) higher levels of citrate, acetone, 2-butanone, proteolytic activity, lactate, ethanol, propanol, formate, moisture and propionate while lower levels of acetate, 2-pentanone, pyruvate, pH and salt content were observed in "bad" Cheddar cheese as opposed to "good" Cheddar cheese. In addition, fine-flavored Cheddar cheese had low concentration of the short-chain FFA. All cheese made with psychrotroph-treated milk had significantly (P < .05) higher short and long chain FFA.

By using stepwise regression as in "Parts I and II" and their variables on 0, 5 and 30 d, it is possible to predict with a fair degree of accuracy the quality of the Cheddar cheese at 180 d.
Conclusions, based on data collected in this study, are as follows:

1.) Control cheese had significantly superior flavor, body and texture than cheese made from the contaminating flora (*E. aerogenes* and *P. fluorescens*), high moisture and bulk starter with low bacterial activity. In addition, the predominant flavors of 180 d cheese made with psychrotroph- and coliform-treated milk and high moisture curd were bitter and unclean.

2.) The yield of the control cheese was significantly higher than that made with psychrotroph-treated raw milk.

3.) All cheese made with psychrotroph-treated milk had significantly higher short and long chain free fatty acids than the control. In addition, fine-flavored Cheddar cheese had low concentration of the short-chain FFA.

4.) Young Cheddar cheese contained low concentrations of FFA and aged desirably-flavored Cheddar cheese had intermediate concentrations of individual FFA.

5.) The aroma and flavor of Cheddar cheese are attributed to a delicate balance of organic acids and volatile organic chemicals produced as metabolites by culture bacteria during fermentation. Overproduction of a particularly desirable flavor
compound may create an imbalance in the overall flavor which is undesirable, e.g. extensive lactic acid or alcohol production impairs cheese quality.

6.) Regression equations were established from the data which laid the groundwork for further study in the area of flavor prediction.

7.) The data established a need for additional study of new and more sensitive techniques for measuring protein degradation and the development of bitter flavor by the production of bitter peptides in Cheddar cheese.
REFERENCES


Perry, K. D. 1961. A comparison of the influence of Streptococcus lactis and Streptococcus cremoris starters on the flavour of cheese. J. Dairy Res. 28:221.


VITA

Najim Hadi Najim was born in Baghdad, Iraq on December 11, 1952. He graduated from Kadhumia High School, Baghdad, Iraq in 1972.

In September, 1972 he entered the University of Baghdad. He graduated with a B.S. degree in Veterinary Medicine in June, 1977.

In September, 1979 he entered the Graduate School of Washington State University in Pullman. He graduated with a M.S. degree in Food Science and Technology in May, 1982.

He entered the Graduate School of Louisiana State University in Baton Rouge to pursue the degree of Doctor of Philosophy in Dairy Science. He was initiated into Gamma Sigma Delta, National Honor Society of Agriculture in March, 1983.

He is now a candidate for the Doctor of Philosophy degree in May, 1985.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Najim H. Najim

Major Field: Dairy Science

Title of Dissertation: The effect of psychrotrophic bacterial contamination on the quality of Cheddar cheese.

Approved:

Charles H. White
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Carranza R. Hackney

D. S. King

Arnold M. Shier

Daniel Ho Hwang

Ronald H. Sneth

Date of Examination:

March 29, 1985