THE EFFECTS OF LACTIC CULTURE SEEDING OF RAW MILK ON THE YIELD AND QUALITY OF CHEDDAR CHEESE

By

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ABSTRACT

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The seeding of raw milk with lactic cultures has been suggested as a method for the inhibition of psychrotrophic bacteria during storage. In this study, 0.5% of a commercial lactic culture composed of a mixture of Streptococcus cremoris and Leuconostoc cremoris strains was used to seed raw milk from the MSU dairy farm. The seeded milk and a control were stored at 3.3 C for 5 days. One-third of each lot was pasteurized and used in the manufacture of Cheddar cheese on the first, third and fifth days of storage.

The milk used in the study had low psychrotrophic bacteria counts precluding any evaluation of this particular commercial culture as an inhibitor. Component analyses of the milk showed slight increases in the acidity in seeded milks.

The cheeses were sampled at 0,6,12,24,36 and 48 months. Component analyses showed non-casein nitrogen content to be the only detectable difference between the control and seeded milk cheeses. Disc-PAGE electrophoresis of caseins showed no detectable differences in gel patterns

between treatments. α_s-Caseins decreased to 50% or less within 6 weeks while β-caseins remained 75-80% intact after 36 weeks of ripening.

Organoleptic evaluation by a consumer panel of 360 individuals found no significant differences in the acceptability of the control and seeded cheeses with over two-thirds of the samples rated as Liked or Sl. Liked on a 5-point Hedonic scale. Few differences were found in the total analysis of the cheese leading to the conclusion that the seeding of raw milk has no detrimental effect on the quality of Cheddar cheese.

To Grandpa and Grandma Palin
for their love and encouragement.

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INTRODUCTION

Shifts in age distribution, economic uncertainty, and the proliferation of nutritional guidelines advocating reduced intake of animal based products have caused the steady decline in per capita sales of many dairy products. One of the bright spots for the industry has been the growth of cheese consumption, which has shown a 53% per capita sales increase from 1970 to 1980 (MIF, 1982). This increased demand for cheese is being supplied despite drastic reductions in the numbers of manufacturing facilities.

Technical advances in the methods of manufacture and in the degree of automation that are used in the cheese plant have enabled the remaining facilities to keep up with demand. But as the cost of labor, raw materials, and energy continue to climb manufacturers are becoming more interested in ways of decreasing costs while maintaining or increasing production levels. One way of achieving this goal would be to increase the yield of cheese from each unit of milk processed, thus creating higher production with essentially the same labor and energy inputs. Or the amount of time that is spent processing each unit of product could be reduced. A third method of cost reduction might be to reduce the number of days worked per week by storing milk

reduce the number of days worked per week by storing milk for future use. This would allow complete shutdown one or two days per week and more efficient use of labor and energy on processing days. Problems arising from the influx of surplus milk in the spring and early summer months could possibly be eased if storage of milk was possible. The ability to spread surpluses out over the plant's normal operating week would increase productivity in a normally hectic time of year. This procedure might also be used throughout the year when situations such as long weekends or inclement weather create short periods of surplus milk. Losses due to low price received for distress milk and excess transportation cost could be avoided if these temporary surpluses could be utilized within the normal processing channel.

Modern dairy facilities are often on 5 day schedules instead of 7 days, therefore, storage of milk is a necessity. If one considers the fact that most milk spends 2 days on the farm and may be stored from Friday until Monday, it becomes apparent that 5 days of storage time might not be uncommon. The detrimental effects on Cheddar cheese yield and quality caused by storing raw milk for prolonged periods of time (>2) days has been demonstrated by Hicks et al., 1980, and Law et al., 1979. They found that high psychrotrophic bacteria counts were the major cause for decreases in yield and quality. Procedures are needed that will reduce losses of yield and quality caused by

psychrotrophic bacterial growth during this time. The addition of lactic bacteria to raw milk in storage has been advocated as a possible means of maintaining the quality of the finished product (Sellars, 1977; White and Shilotri, 1979). The inhibition of psychrotrophic bacterial growth in milk by lactic bacteria has been demonstrated by Price and Lee, 1970, Juffs and Babel, 1975, Babel, 1977 and Pulusani et al, 1979.

Psychrotrophic bacteria are predominant in stored raw milk and have been determined to cause proteolytic and lipolytic breakdown of milk prior to processing (Witter, 1961; Cousin and Marth, 1977; Muir et al., 1978) and during the aging of Cheddar cheese. Law et al., 1976, found that Cheddar cheese manufactured from milk containing lipolytic Gram-negative psychrotrophs developed rancid flavors during the aging process. Heat resistant proteases from psychrotrophic bacteria have been found in milk by Adams et al., 1975, Barach et al., 1976, and White and Marshall, 1972. These heat stable proteases cause bitter off-flavors in milk as well as Cheddar cheese. The problems caused by these psychrotrophic bacteria are especially important in today's dairy industry.

The objectives of this study were:

1. To study the differences in yield and acceptability of American-type cheese resulting from storage of the milk for extended periods of time.
2. To investigate the feasibility of using lactic

cultures as a normal routine for preserving cheese milk when extended storage is required.

3. To investigate the chemical and microbiological differences between the treated milk cheese and the control milk cheese. And to determine which differences, if any, might be correlated with treatment.

LITERATURE REVIEW

Lactic Acid Bacteria in Cheddar Cheese

Identification and Characteristics

The lactic acid bacteria used for the manufacture of Cheddar cheese belong to the family Streptococcacae. They are spherical to ovoid in shape and exist as pairs, chains, and/or tetrads. They are non-motile, non-spore forming, Gram positive, facultative anaerobes which have complex nutritional requirements (Buchanan and Gibbons, 1974). Species from the genera Streptococcus (Str.) and Leuconostoc (Leuc.) are important to the Cheddar cheese industry (Kosikowski, 1977).

The most important bacteria for the manufacture of Cheddar cheese are those belonging to the Lancefield Group N Streptococci. The optimum growth temperature of these organisms is about 30 C and they will grow at 10 C. Streptococcus lactis and Str. cremoris are homofermentative, producing lactic acid. These two organisms may be differentiated on the basis of maximum growth temperature since Str. cremoris will not grow at 40 C but Str. lactis will grow at 41 C. The third member of this group is Str. lactis subspecies diacetylactis (Str. diacetylactis).

Streptococcus diacetylactis is heterofermentative, fermenting citrate in the presence of another fermentable carbohydrate, such as, lactose (Collins, 1971). By-products of citrate fermentation include acetic acid, carbon dioxide and diacetyl (Buchanan and Gibbons, 1974). Citrate alone will not support the growth of Str. diacetylactis (Harvey and Collins, 1963).

Another group of lactic acid bacteria used in Cheddar cheese manufacture is Leuc. cremoris, formerly Leuc. citrovorum. Lactic acid is the major product of Leuc. cremoris but citrate is also fermented to acetic acid, carbon dioxide and ethanol. Leuconostoc cremoris will grow at 10-30 C with optimum growth taking place in a range of 18-25 C (Buchanan and Gibbons, 1974; Sharpe, 1979). Leuconostoc cremoris must be used in combination with other lactic acid bacteria because it does not grow actively until acidic conditions prevail (Drinan et al., 1976). The capability for reducing acetaldehyde to ethanol reduces the chances for a "green" off-flavor produced by some other lactic cultures (Sharpe, 1979). Leuc. cremoris is stimulatory to other cultures making a valuable addition to a starter (Sellars and Babel, 1978).

Lancefield group D streptococci have occasionally been used as starter cultures for hard cheeses (Sharpe, 1979). This group of streptococci is commonly called Enterococci due to the presence of these organisms in the intestinal tract of warm blooded animals. Streptococcus faecalis, Str.

faecium and Str. faecium subspecies durans produce lactic acid in the quantities necessary for cheese manufacture. Many strains of these organisms produce unusually high levels of amines, such as, tyramine and histamine, which may pose health concerns to some consumers sensitive to such compounds (Colonna and Adda, 1976). Tyramine may initiate migraine attacks in some individuals and may produce side effects in individuals using mono amino oxidase to treat hypertension (Law and Sharpe, 1978). Asthma attacks may be intiated by the consumption of histamine by susceptible individuals (Colonna and Adda, 1976). Diebel and Silliker (1963) indicated the role of these enterococci in food poisoning was questionable. However, it is generally recommended that the group D streptococci not be used as cultures for the manufacture of Cheddar cheese (Sharpe, 1979).

Starter Technology

Lactic acid bacteria starters for use in the manufacture of Cheddar cheese may be classified according to the organisms present in the starter and to the method of preparation of the starter for inoculation. Lawrence et al. (1976) in their review of cheese starters stated that three basic types of starters are used worldwide and classified them by bacteriological content. The first type is the Single-strain or Paired strain starter containing one

or two well defined strains of either Str. cremoris or Str. lactis. These starters are most often used in Australia and New Zealand.

Multiple-strain starters made up of three to four well defined strains of Str. cremoris and/or Str. lactis with the possible addition of a strain of Str. diacetylactis and/or Leuc. cremoris are the second type identified. The third type of starters are those containing undefined mixtures of Str. diacetylactis and/or Leuc. cremoris in any combination. These starters are referred to as Mixed-strain starters and are normally found only in the plant of origin and not used for commercial propagation.

The methods of starter preparation have changed a great deal in the past fifteen years. The traditional method for the preparation of starters involved the daily transfer of pure strains in the laboratory. Laboratory "Mother" cultures were then prepared by the inoculation of sterile milk or composite media in bottles with one to four of the pure cultures. Laboratory mother cultures were used to inoculate larger quantities of media, producing "Factory-Mother" cultures. Bulk starters, for the inoculation of cheesemilk, were prepared by inoculation of the required amount of sterile milk or composite media with "Factory-Mother" culture. This process would take as many as four days to complete and require skilled personnel and specialized equipment to prevent contamination (Sandine, 1978; Wilster, 1969). Several new processes for the

propagation and handling of the lactic acid cultures have helped to eliminate some of the costly disadvantages of the traditional methods as outlined above.

Lactic acid bacteria grown in milk will normally grow to a cell density of 10^{8} -10^{9} cfu/ml (Lawrence et al., 1976) before its metabolic products (lactic acid) will limit further culture development. Through the use of buffered media and the chemical and physical control of pH, cells densities of 10^{10} -10^{11} have been obtained (Jespersen, 1979; Sandine, 1977; Osborne, 1977). These cultures may be further concentrated by centrifugation. Starter concentrates produced may then be subjected to either deep-freezing in liquid nitrogen or freeze-drying (lyophilizing). Both processes require a cryoprotective agent, such as, glycerol or sucrose to prevent loss of activity (Lawrence et al., 1976; Sharpe, 1979).

Frozen and lyophilized concentrates have been used successfully to replace "Factory-Mother" culture in the bulk starter process thus eliminating the need for carrying cultures in the local laboratory (Sharpe, 1979). Adding concentrated cultures directly to the vat (DV) has also exhibited success. Salji and Kroger (1979) compared Cheddar cheese made with frozen DV cultures to cheese made with a bulk starter and found no significant differences in the quality of the cheese. Frozen concentrates may be kept 6-8 weeks at -40 C with no loss of activity but at -20 C, they will lose activity in 2-3 weeks (Birkkjaer et al., 1974).

Lyophilized cultures have not proven as successful as frozen cultures for DV use due to the 40-70% loss of viability (Stadhouders, 1969). However, Speckman et al. (1974) found that lyophilized cultures rehydrated in a solution of 20% lactose and 1.0% Stimilac (a product of Marschall division of Miles Laboratories) did not suffer excessive osmotic shock and performed as well as bulk starters in the manufacture of Cheddar cheese. More recently, Vitex of France has developed lyophilized concentrates specifically for DV applications. Normal manufacturing procedures may be used and the resulting cheeses have developed normal flavor and body and texture characteristics (Chapman, 1978; Vassal et al., 1978). If continued success is achieved with lyophilized cultures, they may become more popular because they may be stored at refrigerator temperatures for long periods of time without loss of activity.

Production of Lactic Acid

The production of lactic acid is the most important functional characteristic of the lactic streptococci used as cheese starter cultures. Lactic acid is a product of the fermentation of lactose. Lactose is first hydrolyzed to glucose and galactose. Glucose is metabolized by the glycolytic pathway (Harvey, 1965; Oram and Reiter, 1966) while galactose must first go through the D-tagatose-6P pathway to produce dihydroxyacetone-P and glyceraldehyde-3P

which then join the glycolytic pathway (Bissett and Anderson, 1974). Pyruvate, the final product of glycolysis, is then reduced to lactic acid by lactate dehydrogenase (Anders et al., 1970).

Lactic acid has a wide variety of functions in the manufacture of Cheddar cheese. The initial decrease in pH is important in the coagulation of milk by rennet and other coagulating enzymes. Sellars and Babel (1978) found that as the pH decreased the time required for coagulation decreased, while Rowland and Soulides (1942) observed firmer curd formation with decreased pH. Lactic acid aids in the control of moisture and body and texture through whey and the fusion of curds during the matting process. As a flavor component, lactic acid is very important, especially in fresh curd where it is the major contributor of flavor (Wilster, 1969; Wong, 1974; Kosikowski, 1977).

During ripening, lactic acid continues to play a role even though its production is stopped by the depletion of available lactose. The control of undesirable disease and spoilage causing bacteria is an important function of lactic acid in aging cheese and will be reviewed in a following section. The acidic environment also improves the environment for proteolytic enzyme activity, which contributes to flavor and body and texture development (Wilster, 1969; Wong, 1974; Kosikowski, 1977).

Contributions to the Ripening of Cheese

The roles of both lactic and non-lactic bacteria in cheese ripening have been a topic for study for over 85 years (Babcock and Russell, 1897; Evans et al., 1914; Evans, 1918). It is generally accepted that raw milk cheese ripens faster than pasteurized milk (or starter only) cheese and studies on the contributions of non-starter bacteria are numerous (Alford and Frazier, 1950; Franklin and Sharpe, 1963; Law et al., 1976; Law and Sharpe, 1977). These studies have indicated that raw milk cheeses are also more susceptible to the development of off-flavors. Therefore, the development of an aseptic vat technique (Mabbitt et al., 1959) and subsequent research proving that good quality cheese could be made with starter cultures consisting of only lactic streptococci (Reiter et al., 1967; Law et al., 1976; Law and Sharpe, 1977), have prompted a great deal of research on the role of the lactic streptococci in cheese ripening. This body of research has been extensively reviewed (Mabbitt, 1961; Marth, 1963; Lawrence et al., 1976; Castberg and Morris, 1976; Vedamuthu, 1979; Sharpe, 1979); therefore, this review will not attempt to be all inclusive.

Proteolytic Activity

Early work with proteolysis in cheese indicated that the majority of activity found its source in the bacteria of the cheese (Babcock and Russell, 1897; Peterson et al.,

1948c). More recently, studies have demonstrated that the rennet used for coagulation was responsible for most of the proteolysis (Visser, 1977b; Visser and deGroot-Mostert, 1977) although starter proteases and milk proteases could contribute proteolytic activity on their own. The proteolytic activity of lactic streptococci is quite diverse.

Cell wall-bound extracellular proteinases which degrade caseins to peptides have been isolated for study by Law (1978) and Mills and Thomas (1980). Visser and deGroot-Mostert (1977) demonstrated the ability of lactic streptococcal starters to degrade both α_s- and β-casein in Gouda cheese. β-casein was degraded faster than α_s-casein. Sullivan et al. (1973) and Law et al. (1974) demonstrated that the peptides formed by extracellular proteinases were hydrolyzed by intracellular peptidases.

Studies have also indicated that lactic starters have been responsible for the generation of free amino acids in both Gouda (Visser, 1977b) and Cheddar cheeses (O'Keefe et al., 1976). Lactic starters have demonstrated the ability to utilize free amino acids to produce flavor enhancing derivatives, such as, aldehydes (MacLeod and Morgan, 1958) and volatile fatty acids (Nakae and Elliot, 1965a,b). The proteolytic activity of lactic streptococci was shown to be important to the production of lactic acid by Citti et al.

(1965). They compared slow- and fast-acid producing strains of Str. lactis and found that the fast strain was 4 times as proteolytic.

A problem related to the proteolytic activity of lactic streptococci has been the development of bitter flavor in cheese. Bitter tasting peptides have been identified through knowledge of the solubility of the amino acids in the peptide sequence (Ney, 1971). There are two major theories to explain the formation of these bitter peptides in cheese. The first was set forth by Czulak (1959) and stated that bitter peptides were formed by rennet and certain lactic starters lacked the peptidases necessary to degrade these peptides, allowing them to accumulate in the cheese. This theory has been supported by work done on Gouda (Visser, 1977a,b,c; Visser and deGroot-Mostert, 1977) and Cheddar cheese (Czulak and Shimmin, 1961; Emmons et al., 1962).

The other theory of bitter peptide accumulations was introduced by Lowrie and Lawrence (1972) who stated that all starters could produce bitter cheese if manufacturing conditions were not such that the population of the lactic streptococci was kept low. Support or their work may be found in studies done by Phillips (1935) which showed that all cheeses developed bitter flavors when the pH was 4.90 after 4 days of storage. But, when the pH was greater than 5.00 no bitterness was detected. All cheeses were made from the same starter cultures for comparison. Studies on

Meshanger cheese (deJong, 1978) indicated an increase in diffusion rates of enzymes in high moisture cheese due to increased pore size within the casein matrix. Therefore, moisture should be kept low to prevent excess proteolytic activity which could lead to bitterness.

The argument that high cell densities are the cause of bitterness has been challenged by the work of Visser (1977a) on Gouda cheese. He found no difference in bitterness correlated to cell density. Experiments involving the thermal shocking of cultures also challenge the cell density theory. Pettersson and Sjostrom (1975) and Somkuti et al. (1979) heat treated lactic streptococcal cultures destroying their ability to metabolize lactose but leaving 10-30% of proteolytic activity intact. These thermally shocked cells were then added to the normal starter in concentrations as high as 5 times above normal cell densities with no ill effects on cheese.

Lipolytic Activity

The role of lipid degradation in the flavor of cheese has not been well defined by research. Proper cheese flavor will not result in low-fat or non-fat cheeses (Ohren and Tuckey, 1969), but investigators are not sure if the free fatty acids released by lipolysis are essential to flavor or if the fat is just needed as a solvent or carrier of other flavor components. Patton (1963) found volatile fatty acids to be important to cheese flavor and advocated lipolysis as

the source. According to Peterson et al. (1948a&b) starter lipases did not appear to affect cheese composition until 5 to 20 days of ripening had passed. The total contribution to lipolysis by lactic starters would be small as they are only weakly lipolytic (Reiter et al., 1967). Even if volatile fatty acids have been indicated as important to cheese flavor, it is likely that some of them result from reactions involving amino acids (Nakae and Elliot, 1965a&b; Stadhouders and Veringa, 1973).

Products of Starter Metabolism

All starter streptococci produce variable quantities of acetaldehyde, ethanol, acetic acid, diacetyl, acetoin and volatile acids dependent upon differences in the bacterial strain and cultural conditions (Keenan and Bills, 1968). Hart et al. (1914) found acetic acid, alcohols and esters to be important in the flavor of Cheddar cheese. Typical cheese flavor is made up of a delicate balance of numerous components (Kosikowski, 1977). A lack of or an excess of any one component will have a detrimental effect on the flavor of cheese.

High levels of acetaldehyde give fermented dairy products an off-flavor the industry calls 'green' (Keenan, Lindsay and Day, 1966). This defect has been lessened through the use of selected strains of Str. cremoris, Str. lactis and Leuc. cremoris which have the ability to reduce excess acetaldehyde to diacetyl (Keenan, Lindsay, and Day,

1966; Keenan, Lindsay, Morgan, and Day, 1966). Diacetyl has been shown to be essential to cheese flavor but must also be controlled at levels of less than 0.05 mg per 100 g cheese to avoid an 'acidy' flavor (Calbert and Price, 1949).

The production of excess ethanol by lactic streptococci has been implicated in the production of fruity off-flavors through the formation of ethyl esters (Law and Sharpe, 1977). Bills et al., 1965, demonstrated levels of ethanol 6-16 times greater and levels of ethyl butyrate and ethyl hexanoate 2-10 times greater in cheeses with fruity flavors. The source of these excess components may have been explained by Vedamuthu et al., 1966, when they found excess carbonyls being produced in cheese which had larger than normal numbers of viable cells surviving the manufacturing procedure. The ethanol content of young cheese has also been used successfully to predict fruitiness upon continued ripening (Manning, 1975).

Alteration of the Microenvironment for Chemical Changes

The direct acidification method (Mabbit et al., 1955) was used by Law et al. (1976) to manufacture Cheddar cheese with starters treated with lysozyme. The cells produced no acid and lysed when salt was added to the curd, releasing enzymes thought to be important in ripening. The cheeses made in this manner did not develop Cheddar flavor, leading to the conclusion that intracellular starter enzymes were not directly responsible for production of flavor.

Concentrations of methanethiol were very low in comparison to normal cheese. A good correlation between methanethiol and the intensity of cheese flavor has been demonstrated (Keeney and Day, 1957; Manning, 1975; Manning et al., 1976; Manning, 1978). Law et al. (1976) concluded that the lack of cheese flavor was most likely due to the difference in physical and chemical properties associated with directly acidified cheese. They found that the directly acidified cheese had an oxidation-reduction potential (Eh) of +300 mv, while normal starter activity lowers the Eh to -150 to -200 mv (Frazier and Whittier, 1931).

Kristoffersen (1967) demonstrated an Eh higher than normal associated with cheeses possessing rancid, fermented, and oxidized off-flavors while an Eh lower than normal could result in unclean and sulfide flavors. The active sulfhydryl groups of compounds like methanethiol, which are important to flavor, have an Eh of about -150 mv at pH 5.5. Therefore, as the Eh increases the number of available sulfhydryl groups diminishes, causing a lack of flavor. By lowering the Eh of starter-free, directly acidified cheese, a cheese-like flavor developed which was lacking diacetyl flavor. Therefore, the major role of lactic streptococci may be the alteration of the Eh to a value favoring chemical reactions involving compounds like methanethiol (Manning, 1979).

Problems Associated with the Cold Storage of Raw Milk

Bulk-milk coolers were introduced to U.S. dairy farms in 1937 and have now become the accepted method for storage of milk on the farm. Every-other-day (EOD) pickup is the industry standard and tanks used for this purpose must have the ability to cool 25 percent of their total capacity to 10 C within 1 hr and to 4.4 C within 2 hr of the end of milking (Campbell and Marshall, 1975). Strict hygiene during milking and storage is also needed to provide a low probability of contamination by adventitious bacteria. The temperature of the milk should not exceed 4.4 C at any time during transfer to the processing facility or during subsequent storage (Campbell and Marshall, 1975; Thomas and Druce, 1971).

Storage of raw milk at the plant is most commonly accomplished in silo-type tanks which may or may not be refrigerated. The quality of the raw milk at the end of the in-plant storage period is directly related to [1] the initial temperature, [2] the bacteriological quality of the product, [3] the cleanliness of the storage vessel and related transfer equipment and [4] the length of the storage period [Roberts, 1979; Campbell and Marshall, 1975]. Even though the latest in storage equipment and the best sanitation procedures may be in use, there are still problems with the quality of raw milk and its subsequent products when stored for extended periods of time. These

may be related to the endogenous components of raw milk or to the exogenous components introduced by bacterial growth and metabolism.

Endogenous Components

Milk is a very complex biological system containing proteolytic and lipolytic enzymes which may affect the quality of the raw milk and/or the finished products. A complex structure of some components also makes milk vulnerable to alteration during storage.

Proteolytic Activity

Endogenous proteolytic activity in milk has been demonstrated in many studies over the last 85 years (Babcock and Russell, 1897; Warner and Polis, 1945; Storrs and Hull, 1956; Harper et al., 1960; Noomen, 1975). Until recently, the effects of these enzymes has been considered unimportant to the quality of milk and its by-products (Harper et al.,1960; Zittle, 1964).

The principle enzyme has traditionally been referred to as alkaline milk protease, but in recent years research has lead to the conclusion that it is very similar or identical to blood plasmin (Eigel, 1977; Reimerdes et al., 1981; deRham and Andrews, 1982). Optimum conditions for the activity of this enzyme in milk have been reported as a temperature of 37-45 C and a pH of 6.5-9.0 (Humbert and Alais, 1979). Milk plasmin exhibits specificity for the

degradation of both α_{s}-casein and β-casein. Noomen (1975) showed that β-casein was degraded at 2 to 3 times the rate of α-casein. The degradation of β-casein has been found to be responsible for the appearance of the γ-caseins and the 5-and 8-fast components of the proteose-peptone fraction of milk (Mercier et al., 1972; Visser and de Groot-Mostert, 1977; Andrews, 1978 a,b; Jenness, 1979). Chen and Ledford (1971) found no evidence for the degradation of κ-casein by milk plasmin.

Kaminogawa and Yamauchi (1972) demonstrated the presence of an acid proteinase in milk with an optimum activity at pH 4.0. More evidence for the presence of acid proteinase in milk was given by Noomen (1978a). He found that α_{s1}-casein was degraded more rapidly than β-casein in Meshanger cheese at a pH of 5.4, indicating the presence of a proteinase other than plasmin.

The stability of milk proteases to heat treatment has been well documented. Little or no activity was lost at pasteurization time and temperature combinations in studies by Storrs and Hull (1956), Harper et al.(1960) and Creamer (1975). Pasteurization of milk at 75 C for 15 sec and 63 C for 30 min resulted in 30-40% and 8-24% increases in milk protease activity, respectively (Noomen, 1975). These increases were thought to be due to either favorable modification of the substrate or inactivation of a specific protease inhibitor. Later work by deRham and Andrews (1982) indicated that the increase in activity noted by Noomen may

have been due to increased activation of plasminogen to plasmin. The increased activation seemed to involve destruction of an activation inhibitor or activation of an activation promoter.

The effects of endogenous milk proteases on raw milk during storage is not normally of concern unless there are abnormally high levels of proteases due to high somatic cell counts (Ali et al., 1980b). Proteolytic activity from endogenous enzymes has been demonstrated in Meshanger cheese (Nooman, 1978 a,b), Gouda cheese (Visser, 1977 a,b; Visser and deGroot-Mostert, 1977) and Cheddar cheese (Green and Foster, 1974). In all cases these investigators concluded that milk protease contributed to cheese ripening but to an insignificant degree when compared to rennet and bacterial proteases. Visser (1977a) also concluded that milk protease made no contribution to the bitter off-flavor of Gouda cheese.

Lipolytic Activity

The presence of lipases in milk as it is drawn from the udder has been well established (Palmer, 1922; Roadhouse and Henderson, 1935; Hunter et al., 1968). Lipase enzymes in milk are responsible for the hydrolysis of milk triglycerides, thus releasing fatty acids into the milk, imparting a characteristic flavor termed 'hydrolytic rancidity'. The activity and characteristics of these lipases have been reviewed by Schwartz (1974), Deeth and

Fitz-Gerald (1976) and Downey (1980). Therefore, only brief mention will be made of endogenous lipases as they affect raw milk in storage and its final products.

Two types of lipolytic activity are generally attributed to endogenous lipases. Normal co-mingled, raw milk will not develop the rancid flavor if properly cooled and handled, but 'induced' rancidity may develop if proper handling conditions are not maintained. Rancidity may be induced by any physical treatment that will disrupt the fat globule membrane which protects the fat globule under normal raw milk conditions. Physical mistreatment of the raw milk may include violent agitation, foaming, temperature fluctuations, homogenization, and freezing and thawing.

'Spontaneous' rancidity is the other type of 'hydrolytic rancidity' normally found. Milk produced by a small percentage of cows will exhibit a susceptibility to the formation of rancid flavor (Roadhouse and Henderson, 1935; Hunter et al., 1968, Downey, 1980). The only treatment necessary to produce spontaneous rancidity is the cooling and storage of the raw milk, which will develop the off-flavor within 48 hr even under quiescent storage. If spontaneous milk is mixed with normal milk in a ratio of at least 1:4 then the spontaneous milk will not affect the quality of co-mingled milk. Factors increasing the probability of spontaneous rancidity include late lactation, low energy and high carbohydrate feeds, low milk production, and the 'heat' portion of the oestrus cycle.

Pasteurization has generally been accepted as the proper procedure for the elimination of the lipase activity (Roadhouse and Henderson, 1935, Deeth and Fitz-Gerald, 1976, Schwartz, 1974. However, Schwartz (1974) reported that Nielson and Willort and Harper and Gould showed a 10-20% residual lipase activity following pasteurization of normal raw milk.

Chemical and Physical Changes

Enzyme activities are not the only endogenous influences on the quality of raw milk and its by-products. Chemical and physical changes may also affect product quality. The development of oxidized flavor, a chemical reaction, in dairy products has been reviewed by Parks (1974). He reported the classification scheme of Thurston for milk based on oxidation susceptibility. First, spontaneous milk is that which exhibits oxidized flavor within 48 hr of storage. Second, susceptible milk is that which exhibits oxidized flavor within 48 hr after contamination with copper, a strong pro-oxidant. And third, resistant milk is that which will have no oxidized flavor after 48 hr even with the addition of copper. Susceptibility for oxidation is generally accepted to be a function of the percentage and/or distribution of naturally occurring pro-and antioxidants (Parks, 1974). The use of

proper cleaning and sanitizing procedures and approved materials for milk handling have greatly reduced the incidence of autoxidation.

A physical change in milk that has great impact on its suitability for the manufacture of cheese is the dissociation of caseins from the micelle. Kolar and Brunner (1967) and Rose (1968) studied the serum caseins and found that β-, κ- and α_s-caseins were all present in the serum fraction of milk in decreasing concentrations, respectively. Ten to fifteen percent of the total casein was found in the serum and the amount of serum casein increased with decreasing temperatures (Rose, 1968; Downey and Murphy, 1970). All caseins have shown endothermic association in milk or in purified systems (Payens, 1966). β-casein accounted for 43% (Downey and Murphy, 1970) or 55% (Rose, 1968) of the increase in serum casein due to decreasing temperatures. Studies by Payens and van Markwijk (1963) and Garnier (1966) showed that caseins went through conformational changes when the temperature was raised from 5-40 C. Included in the change was the formation of β-casein polymers as the temperature rose, paralleling the association characteristics of the casein micelle.

The caseins present in the serum of milk have been shown to be more susceptible to proteolysis than those in the micelle (Ledford et al., 1968). Ali et al. (1980a) studied the dissociation of the casein micelle during storage of milk for cheese and found decreased yields,

weaker curd formation, longer renneting times and increased fat losses as the amount of casein in the serum increased. They also found that the β-casein in the serum increased for 48 hr at 4 or 7 C but further storage and heat treatment at 60 C for 30 min brought about partial reversals of the casein dissociation. Yields of cheese manufactured from milk stored 72 hr at 4 C were higher than yields from milk stored 48 hr at 4 C.

Exogenous Components

Exogenous influences on the quality of raw milk in storage are mainly of bacterial origin. There are four types of bacteria commonly found in raw milk. They are psychrotrophic, mesophilic, thermophilic and thermoduric. Psychrotrophic bacteria are those that will grow at a relatively rapid rate at or below 7.2 C and are capable of slow growth at temperatures as low as O C (Pelczar et al., 1977; Witter, 1961). The majority of these are Gram-negative, non-spore forming, aerobic rods which are nearly all sensitive to heat (Blankenagel, 1976; Boyd et al., 1953). The largest group of organisms in milk are the mesophilic bacteria. Those bacteria that grow best within the temperature range of 25-40 C are in this group. This group is seldom a problem in the dairy industry because pasteurization destroys most of them and cold storage temperatures of 4 C or below are not conducive to growth (Pelczar et al., 1977).

Another group of organisms found in raw and pasteurized milk are the thermophilic bacteria. Thermophiles grow well at temperatures above 50 C. Although these organisms may survive pasteurization, their ability to grow at refrigeration temperatures is so limited that they should not be a problem (Pelzar et al., 1977). The fourth group of organisms found in raw milk are the thermoduric bacteria. Thermodurics may not grow at pasteurization temperatures but can survive the treatment (Pelczar et al., 1977). Molska et

al. (1977) reported destruction of 22.1, 42.4, and 77.1 percent of the original thermoduric population at pasteurization temperatures of 72 C, 76 C, and 84 C, respectively, for fifteen seconds. These organisms may belong to any of the other three classifications, depending on their optimum growth temperature. Witter (1961) reported the results of several studies that found psychrotrophs with thermoduric capabilities. Work by White et al. (1978) reported on a milk plant that was receiving milk containing thermoduric psychrotrophs. Most of these bacteria are Gram-positive spore formers, such as species of the Bacillus genus, but may include species of the Microbacterium, Micrococcus, and Streptococcus genera (Molska et al., 1977; Pelzar et al., 1977). They can be isolated from dirty utensils, feed, manure, soil, water, and the ducts of a cow's mammary gland.

Boyd et al. (1953) in a study of the keeping quality of commercially pasteurized milk reported that milk with the longest shelf life was eventually spoiled by thermoduric bacteria. In the past, thermoduric bacteria were accepted as the spoilage organisms in pasteurized milk that was free of post-pasteurization contamination (Hall and Trout, 1968). This theory can be debated. A study of pasteurized milk reported by Molska et al. (1977) found acid-producing and caseolytic bacteria that were not considered thermodurics. The destruction rates were 99.7, 99.9, and 100 percent for the acid producers and 88.4, 92.9, and 99.8 percent for the

caseolytic bacteria at 72 C, 76 C, and 84 C, respectively, for fifteen seconds. They concluded that sufficiently high contamination of the raw milk supply could lead to spoilage of pasteurized products by non-thermoduric bacteria.

The introduction of modern refrigeration has emphasized the importance of psychrotrophic organisms to the dairy industry and many reviews on the subject have been written. Witter (1961) reviewed these organisms when they were called psychrophilic or "cold-loving" bacteria. By the time Thomas and Thomas (1973a,b) reviewed the subject the term psychrotrophic or "cold-thriving" bacteria was in general use in the dairy industry. The term psychrotroph, appeared more appropriate based on the fact that most of the organisms involved have optimum growth temperatures well above 7.2 C. Extensive literature reviews on the organisms involved and the results of their activity in milk have been reported by Mikolajcik (1979, 1980a,b) and Cousin (1982).

While the majority of psychrotrophs identified have been Gram negative rods, such as Pseudamonas, Alcaligenes, Aeromonas, and Flavobacterium, many Gram positive bacteria, such as Micrococcus, Bacillus, Arthrobacter, and Streptococcus have also been identified. Thermoduric psychrotrophs are found in the latter group which contains many sporeformers (Cousin, 1982). The psychrotrophic portion of the total bacterial population is variable as reported by Gehringer (1981) who found that psychrotrophs varied from 9.5-98.8% of isolates from 32 different suppliers.

Pseudomonas species are the most commonly encountered of the psychrotrophic organisms. Richard (1981) found that 90% of the psychrotrophic isolates from milk stored at 4-5 C for 4 days were Pseudomonads even when they constituted only a small percentage of the original population.

A population of 10^6 bacteria per ml of milk has been generally accepted as the level at which changes in the milk become measurable (Cousin, 1982; Marshall, 1982). Typical generation times for psychrotrophs of 5.5 to 10.5 hr at 4 C have been demonstrated (Richard, 1981; Mabbit, 1981). Therefore initial population of 10^4 bacteria per ml would easily yield a population over the 10^6 bacteria per ml limit on the third day of storage, which points out one of the difficulties encountered in long term raw milk storage. Most of the damage to milk and its products caused by psychrotrophs has been attributed to the production of proteolytic and lipolytic enzymes produced by many of these organisms. Detailed reviews of the enzyme activities have been published by Law (1979), Muir et al. (1979), Mabbitt (1982) and Cousin (1982). This review will only highlight some of the pertinent research findings.

Results of a study in Finland showed that raw milk stored for 4 days at 4 C had a 5.5% reduction in the proportion of casein nitrogen to total nitrogen, indicating a breakdown in casein which was correlated with an increase in the psychrotrophic count to 10^7 per ml (Antila, 1982). In work done by Knoop and Peters (1979) raw milk for cheese

held at 10-12 C for 78 hr was slower to coagulate than milk held at 4 C for 78 hr. This problem was blamed on the activity of proteases on the micellar casein.

Adams et al. (1975) found that 70-90 percent of the samples they collected contained psychrotrophs capable of producing heat-resistant proteases. These proteases were capable of hydrolyzing α_s-, β- and κ- casein. Of special interest was the significant level of hydrolysis of κ-casein when the population was only 10^4 psychrotrophs/ml. Destruction of these proteases required a time-temperature treatment of 90 sec at 140 C. With this treatment, milk caseins would be adversely affected. Attempts by Barach et al. (1976) to inactivate heat-resistant proteases at lower temperatures or shorter times did not prove effective. Holding the milk at 149 C for only ten seconds eliminated most of the casein denaturation problem but reduced protease activity by only 30%. Temperatures below 50 C showed no inactivation of heat-resistant proteases. Seventy percent inactivation was observed at 55 C for sixty minutes but that procedure proved too time-consuming to be of practical use. Richardson (1981) found an extracellular heat resistant protease from Ps fluorescens B52 with a half-life of 37.5 sec at 150 C. Heat resistant proteases have been demonstrated in cultures of Ps. fluorescens P26 which required 9 min at 121 C for destruction of activity (Mayerhofer et al., 1973; White and Marshall, 1972).

White and Marshall found significantly higher levels of proteolysis and lower flavor scores in Cheddar and Cottage cheese made with milk that had Pseudomonas fluorescens P26 added to it, twelve hours before pasteurization. The protease from P. fluorescens P26 retained 71 percent of its activity after 60 min at the pasteurization temperature of 71.4 C. Work done at the University of Wisconsin demonstrated proteolytic activity in milk through increased levels of non-casein and non-protein nitrogen in cheese milk precultured with psychrotrophic bacteria (Cousin and Marth, 1977b). They also found that the precultured milk coagulated faster upon the addition of rennet than did the control milk. The curd was of a firmer body resulting in lower losses of milk solids in the whey. They proposed that the proteases had modified the casein making it more susceptible to the action of rennet. The slightly higher level of acid in the precultured milk may also have contributed to speed of coagulation.

Muir et al. (1978) did not find a significant correlation between total psychrotrophic counts and rancidity in milk but did find that no rancidity was observed when psychrotrophic counts were below 5×10^6 bacteria per ml. This lack of correlation was likely due to the fact that psychrotrophs are not all actively lipolytic. Mabbitt (1981) in his review concluded that one of the major contributions of psychrotrophs to rancidity in milk was the production of phospholipases which attack the lipoprotein

complexes of the fat globule membrane. This activity exposes the rest of the fat globule to the action of endogenous lipases resulting in rancid flavors.

The ability of lipases produced by psychrotrophs to survive heat treatment and cause product damage has been demonstrated in two studies involving the manufacture of Cheddar cheese (Cousin, 1976; Law et al., 1976). Both studies used milk that had been precultured with psychrotrophic bacteria and then pasteurized. Rancid flavors developed in all of the cheeses treated in this manner.

Although the enzymes of psychrotrophs are the most important to product spoilage, it is also possible that high growth rates in milk stored for longer periods of time might cause the accumulation of metabolites. Hosono et al. (1974) found that some psychrotrophic bacteria produce 10 times more ethyl butyrate and ethyl hexanoate than lactic streptococci. This may lead to fruity off-flavors in the milk and its by-products.

Control of Psychrotrophic Bacteria

Methods of controlling the growth of psychrotrophic bacteria reported in the literature include refrigeration, heat treatment, chemical additives and bacteriological cultures (Witter, 1961; Mikolajcik, 1980a; Cousin, 1982). Although the advent of refrigeration enhanced the importance of this group of bacteria it can also be used to help control growth. Generation times for several of the most frequently isolated varieties of Ps. fluorescens were found to increase from a range of 5.5-10.5 hr at 4 C to a range of 12.5-16.5 hr at 1 C (Richard, 1981). This study demonstrated the ability to hold milk as long as 7 days at 1 C without significant reduction in quality. In his study of farm milk supplies, O'Conner (1981) demonstrated that the psychrotrophic count of milk stored at 2 C for 4 days was the same as the count of a duplicate sample held at 4 C for 3 days.

Control of psychrotrophs with heat treatments may take the form of either a sub-pasteurization treatment, called thermization, or pasteurization. Thermization is a European process used to prevent further deterioration of the product when stored at the processing facility. Time-temperature relationships of 63-66 C for 15 sec were reported by Cousins et al. (1977) and Zall (1980). Stadhouders (1982) reported a process using 64-68 C for 10 sec. He also reported that extra precautions must be taken to avoid post-treatment contamination and that the heat exchanger may require more frequent cleaning due to the growth of thermophilic organisms, such as Str. thermophilus. One benefit of this procedure seemed to be the activation of Bacillus cereus spores making them susceptible to destruction by the pasteurization process. Research at Cornell University has centered on heat treating milk at the farm prior to cooling (Zall and Chen, 1981). A heat treatment of 74 C for 10 sec has been used and milk treated in this manner has shown no deterioration over 7 days storage at 3 C.

Pasteurization eliminates most of the psychrotrophic bacteria found in milk. Therefore high psychrotrophic counts in pasteurized milk are usually considered to be due to post-treatment contamination (Witter, 1961; Cousin, 1982). Partridge et al. (1982) stored pasteurized milk in bulk for up to 10 days with no significant decrease in the quality of Mozzarella cheese. Pasteurization should not be used as a substitute for good cleaning and sanitation

practices because the efficiency of pasteurization may be reduced from 99% bacteria kill to as low as 50% kill if the initial bacterial count is high enough (Hall and Trout, 1968; Davis, 1975).

Chemical treatments that have been used to control psychrotrophs include hydrogen peroxide-catalase, carbon dioxide nitrogen and hydrogen peroxide-thiocyanate. Hydrogen perioxide (H_2O_2) -catalase treatment has proven to be an acceptable preservation technique in situations where refrigeration is not available (Lampert, 1970). It should not however be considered a substitute for pasteurization because it may not eliminate all pathogenic organisms. Hydrogen peroxide is the active ingredient while catalase is added to catalyze the degradation of residual H_2O_2. The hydrogen peroxide-catalase treatment has been approved in the U.S. for use in the manufacture of Cheddar cheese, Colby, washed curd, granular and Swiss cheeses (Wilster, 1969). More starter and color may be required in the manufacturing process but otherwise processes are unchanged. Cheesemakers like this procedure because it does not destroy the endogenous enzymes. The use of too much H_2O_2 causes bitter, high moisture defects in cheese (Lampert, 1970). Fox and Kosikowski (1962) found that Cheddar cheese made from H_2O_2-catalase treated milk had a bland and somewhat foreign flavor.

Carbon dioxide (CO_2) has been used for many years to control microorganisms in carbonated beverages. Witter

(1961) reported that research involving the carbonation of milk was unsuccessful but that CO_2 has been successfully used to replace oxygen in package headspaces (Kosikowski and Brown, 1973; Tsantilis and Kosikowski, 1960). Mabbitt (1982) revived the possibility of using CO_2 as a bulk storage preservative. He carbonated raw milk and pressurized the tank atmosphere with CO_2, then held the milk for 7-8 days at 4 C before counts reached 10^6 bacteria per ml. The pH of the milk dropped to 6.0 upon carbonation but returned to normal when the CO_2 was removed by warming the milk in a reduced pressure vessel. The procedure inhibited psychrotrophs but did not inhibit lactic acid bacteria. Problems left to overcome include some localized casein precipitation during the addition of either gaseous or solid CO_2.

Another gas that has been successfully employed as a package flushing agent is nitrogen. Nitrogen extended the shelf-life of cottage cheese in studies by Kosikowski and Brown (1973) and by Tsantilis and Kosikowski (1960). Recent work in Canada has demonstrated no detectable levels of proteinase activity in raw milk flushed with nitrogen at a flow rate of 100 ml/min (Murray et al., 1983). This reduction in proteinase activity was observed despite the fact that the growth of proteolytic psychrotrophs was not inhibited.

A treatment involving the addition of hydrogen peroxide and thiocyanate (SCN^-) utilizes the natural lactoperoxidase

system of milk. The presence of a peroxidase in milk has been known since the work of Arnold in 1881 (Johnson, 1974). The lactoperoxidase system has been reviewed by Bjorck (1982) and Reiter and Harnulv (1982a,b). Lactoperoxidase catalyzes the oxidation of SCN^- by H_2O_2 giving rise to the intermediate products, cyanosulphurous/cyanosulphuric acid and hypothiocyanate. These intermediate products have shown a broad spectrum of activity against Escherichia coli, Pseudomonas spp., Salmonela typhimurium, Streptococcus cremoris, Bacillus cereus and many other Gram negative and positive organisms. The eventual lysis of the cells is a consequence of inhibition of glycolysis, reduced uptake of carbohydrates and amino acids and leakage of potassium from the cell. The system requires the addition of 8-10 parts per million of H_2O_2 and the 1-15 parts per million NaSCN. Any residual H_2O_2 or intermediates are destroyed during subsequent pasteurization and the amounts of additives are equivalent to levels present in milk under the optimum physiological condition of the cow. Both of the aforementioned reviewers have endorsed the procedure for underdeveloped countries which lack refrigeration at the farm level.

Successful trials of the system have been carried out in Sri Lanka (Harnulv and Kandasamy, 1982) which increased the usable life of the unrefrigerated milk by several hours. The effects of the lactoperoxidase system on cultured products was investigated by Zall et al. (1983b).

They found that yogurt could be made successfully but buttermilk developed bitter off-flavors. Cheddar cheese was also manufactured from lactoperoxidase treated milk and resulted in lower yields, slow acid production, and slow, though unobjectionable, ripening (Zall et al., 1983a).

Four types of inhibitory activity have been identified in lactic streptococci. The first three are antibiotics, hydrogen peroxide, and organic acids which are produced during the growth of the bacteria and the fourth is nutrient competition which is due to their fast rate of growth.

Nisin is a polypeptide of about 3500 daltons which is produced by some strains of Str. lactis. It was recognized as an antibiotic in 1944 by Mattick and Hirsch. Nisin has a fairly broad spectrum of activity which includes activity against most streptococci. Some Staphylococci, Micrococci, Pneumococci, Neisseria, Bacillus, Clostridium, Mycobacterium, Lactobacillus, Actinomyces and Erysipelothrix (Mattick and Hirsch, 1949). The strength of activity varies with the organism but none are inhibited as strongly as Str. cremoris.

Diplococcin is also a polypeptide that was recognized as an antibiotic in 1944 by Oxford. It is produced by Str. cremoris and has a molecular weight of about 5300. It has a narrow spectrum of activity which includes the group N (lactic) streptococci and only transigent effects on Staph. aureus. In a study by Davey and Richardson (1981) only 11 of 150 strains assayed produced detectable quantities of

diplococcin. Neither nisin or diplococcin have any activity against Gram-negative coliform type bacteria, thus limiting their effectiveness against psychrotrophs.

The production of hydrogen peroxide by lactic streptococci was demonstrated in a study by Gilliland and Speck (1969) in which catalase was utilized to determine if autoinhibitory levels of H_2O_2 were being produced. A study by Haines and Harmon (1973) demonstrated that greater numbers of Staphylococcus aureus were able to grow in the presence of Str. lactis when the media were treated with catalase, indicating H_2O_2 as the inhibitor. As little as 5 µg H_2O_2/ml of product has proven effective in inhibiting bacterial growth.

The major product of lactic streptococci is lactic acid which has been shown to have some inhibitory activity. Amster and Jost (1980) found that E. coli were inhibited more at pH 5.0-5.3 with lactic acid than at pH 4.5 with HCl. Str. diacetylactis and Leuc. cremoris also produce acetic acid which has proven to be very toxic to undesirable bacteria (Pinheiro et al., 1968; Sorrells and Speck, 1970; Gilliland and Speck, 1972). Chandan et al. (1977) demonstrated the development of benzoic acid by lactic cultures in cultured dairy products, such as Cheddar cheese. The inhibitory activity of these and other organic acids produced by lactic streptococci probably have an additive effect that is not limited to the effects of pH.

Nutrient competition has been demonstrated as a means of inhibiting the growth of spoilage organisms in cottage cheese. Elliker et al. (1964) found that inhibition of spoilage organisms was directly related to the population of Str. diacelylactis in the finished product.

Juffs and Babel (1975) have studied the inhibition of psychrotrophic bacteria in milk in cold storage by the addition of 0.5% of 5 commercial lactic cultures numbered 8, 44, 70, 80 and 95. By comparing the inhibitory effects of the lactic cultures, the importance of selection for inhibitory abilities was pointed out. They found that the degree of inhibition varied with the culture used, the initial population of psychrotrophs, the type of psychrotroph, the storage temperature and the time. The inhibition consisted of a reduction in growth rate rather than population and was decreased by catalase treatment suggesting H_2O_2 as the inhibitor.

It is also possible that acid levels contribute to inhibition over the storage time. Decreasing pH may also help in reducing the manufacturing time for cheeses.

White and Shilotri (1979) used a 0.5% inoculum of Str. diacetylactis in combination with Leuconostoc cremoris to treat raw milk in the farm tank. They found no statistical differences in psychrotrophic or coliform counts but did find an increase of 1 day in the shelf-life of finished products.

Rutzinski and Marth (1980) studied the inhibition of some coliform organisms by Str. lactis, Str. cremoris and commercial starters. Str. lactis produced reductions in Hafnia sp. populations, with a 2.0% inoculation virtually eliminating the Hafnia after 12 hr. Str. cremoris produced similar reductions in pH values as Str. lactis but inhibition of the Hafnia organisms was not as pronounced. All Str. cremoris inoculations and incubation temperatures allowed increased populations after 15 hr.

One of the commercial cultures used produced less acid under the conditions of the experiment than either the Str. lactis or Str. cremoris cultures. Inhibition was also lower than encountered with the other cultures, thus indicating the importance of acidity. These experiments reinforce the need for careful selection of cultures for use as inhibitors of unwanted bacterial contaminants. They also indicate the possible additive nature of the inhibitory activities since it is apparent that acidity was involved in Str. cremoris activity while acidity plus another factor seemed to be operating in the Str. lactis culture.

Information about antibiosis by organisms other than lactic streptococci has been included in reviews by Babel (1977), Speck (1979) and Chandan (1982).

EXPERIMENTAL PROCEDURES

Treatment of Milk

Milk, for the manufacture of Cheddar cheese, was obtained at the Michigan State University (MSU) dairy research farm. Approximately 844 Kg of milk was picked up after the fourth milking had been added to the farm tank and the product temperature had reached 4.4 C or below. Upon receipt at the MSU dairy plant, the load was divided into control and experimental lots of 422 Kg each. Both the control and experimental lots were stored in stainless steel tanks at 3.3 C.

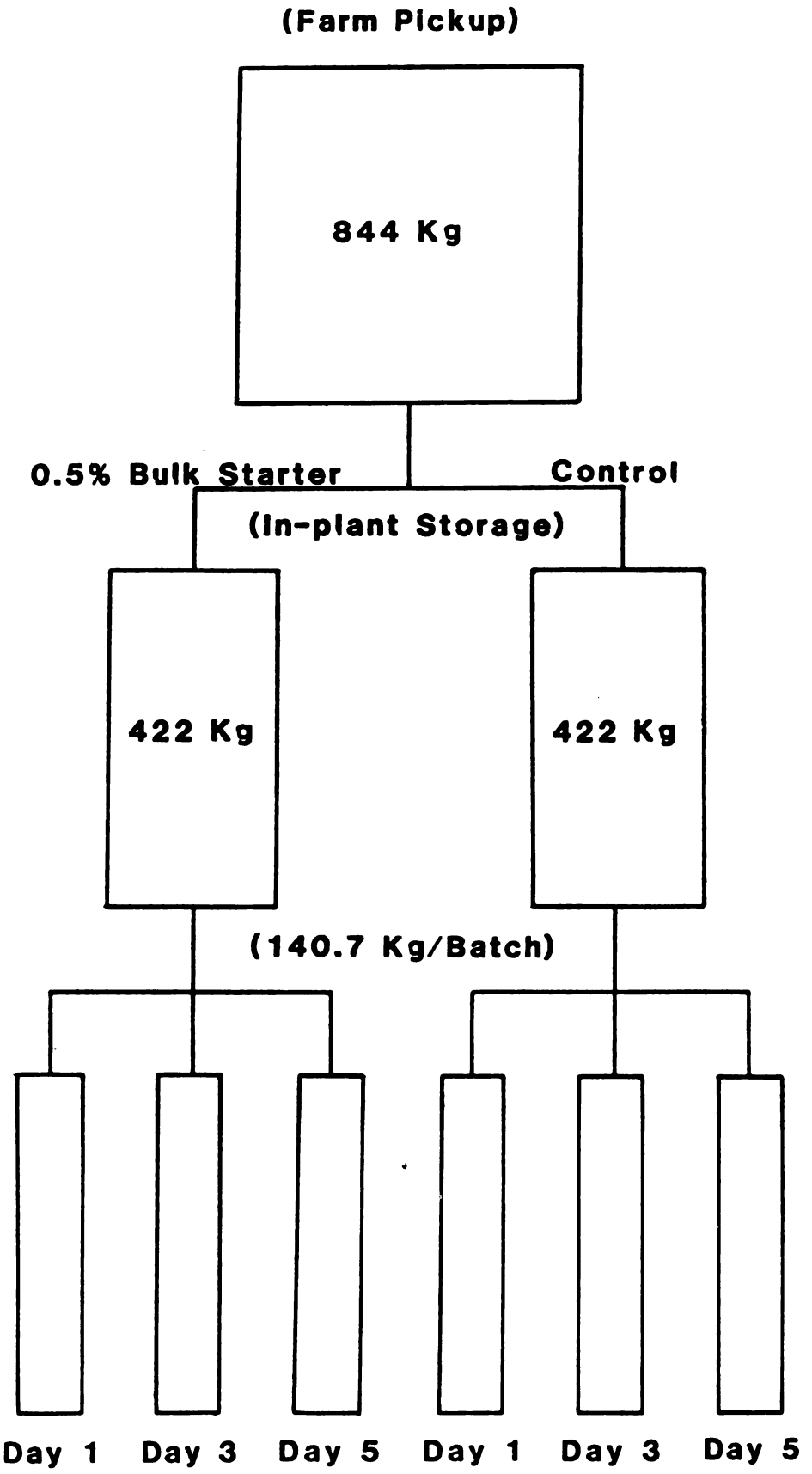
The experimental lot of milk was seeded with 0.5% of a commercial, lactic bulk starter at the time of receiving. One-third (140.7 Kg) of each lot was withdrawn on the first, third, and fifth days of in-plant storage for the purpose of manufacturing Cheddar cheese (Figure 1). The milk for each batch of cheese was pasteurized at 62.8 C for 30 min and cooled to 31 C immediately prior to cheese manufacture.

Four trials were performed yielding a total of 24 batches of Cheddar cheese.

Preparation of Bulk Starter

The bulk starter media was prepared by the addition of 0.44 Kg of low heat, antibiotic free, spray dried skim milk to 3.49 Kg of water to yield a total solids content of 11%. The media was heated to 90.5 C for 60 min and promptly

Figure 1. Flow diagram illustrating the partitioning of milk for experimental and control treatments as well as partitioning for days of storage.


(Farm Pickup)
844 Kg
0.5% Bulk Starter
Control
(In-plant Storage)
422 Kg
422 Kg
(140.7 Kg/Batch)
Day 1
Day 3
Day 5
Day 1
Day 3
Day 5

cooled to 21.1 C for inoculation. The inoculation consisted of 0.7 ml of a frozen concentrated lactic culture. The culture was Redi-Set #253, provided by Chr. Hansen's Laboratories of Milwaukee, Wisconsin, and consisted of a mixture of Streptococcus cremoris and Leuconostoc cremoris. The bulk starter was incubated at 21.1 C until a titratable acidity of 0.70-0.75% developed, usually in 14-16 hr. It was then cooled to 4.4 C or below and used within 12 hr (Sellars and Babel, 1978).

Manufacture of Cheddar Cheese

Cheddar cheese was manufactured according to normal industry practices as outlined by Wilster, 1969. The pH of the curd and titratable acidity (T.A.) of the drained whey were used as indicators for the determination of milling time. A pH of 5.25-5.35 and a T.A. of 0.45-0.50% were used as indicators in order to yield a high moisture cheese capable of rapid ripening.

The cheese milk was set with calf rennet and the same bulk starter used as a seed for the experimental treatment. Curds from each batch were placed in 3 - 9.1 Kg Wilson cheese hoops. After pressing overnight, the blocks were cut in half and each block of approximately 4.55 Kg was vacuum packaged in Cryovac barrier bags. The six cheeses from each batch were labeled and stored in a cold room at 4.4 C for ripening. A manufacturing report (Figure 2) was completed for each batch.

Figure 2. Cheddar cheese manufacturing report.

PLANT REPORT

Check One: Control ☐ Treatment ☐ Date: _____

Milk

B.F. of Milk..... _____%
pH of Milk..... _____
T.A. of Milk..... _____%
Temp. of Pasteurization..... _____F
Duration of Pasteurization..... _____
Time of Pasteurization..... _____
Wt. of Milk in Vat..... _____lbs
Temperature of Milk Going Into Vat..... _____F
Time at Filling Vat..... _____

Starter

pH of Starter..... _____
T.A. of Starter..... _____%
Wt. of Starter Added..... _____lbs
Time at Adding Starter..... _____
Temp. of Milk When Starter Added..... _____F
pH of Milk & Starter Just Prior to Adding Rennet... _____
T.A. of Milk & Starter Just Prior to Adding Rennet. _____%

Rennet

Volume of Rennet Added..... _____ml
Volume of Water for Dilution..... _____ml
Time at Adding Rennet..... _____
Temp. of Milk at Setting..... _____F

Cheese

Time at Cutting..... _____
Time Start Cooking..... _____
Temp. at Start Cooking..... _____F
T.A. at Start Cooking..... _____%
pH at Start Cooking..... _____
Time Finish Cooking..... _____
Temp. at Finish Cooking..... _____F
T.A. at Finish Cooking..... _____%
pH at Finish Cooking..... _____
pH of Curd at Completion of Cheddaring..... _____
pH of Whey at Completion of Cheddaring..... _____
T.A. of Whey at Completion of Cheddaring..... _____%
Wt. of Whey..... _____lbs
Time of Completion of Cheddaring..... _____
Temp. at Completion of Cheddaring..... _____F
Time of Milling..... _____
Time of Salting..... _____
Salt Added..... _____lbs
Pressing Time..... _____
Wt. of Cheese..... _____lbs
Yield..... _____%

Observations:

Weights and Yields

The weight of each batch of milk was recorded immediately prior to pasteurization. The weights of the starter and diluted rennet were also recorded and added to the original weight of the batch for yield calculations. Whey was collected in 37.9 l cans and weighed as part of the materials accounting procedures. Cheddar cheese was weighed at the time of packaging.

The recorded weights were used to calculate the actual wet yield. Weights and total solids values were incorporated in the calculations of yields on a dry weight basis (Table 1).

Organoleptic Evaluation

Trained Panel

A trained panel consisting of three experienced judges used the American Dairy Science Association (ADSA) score card (Figure 3) to evaluate the cheeses after 12 weeks of ripening. Their purpose was to identify possible defects and determine if there were detectable differences in cheeses. When differences were found, the cheeses were subjected to a consumer panel for acceptability and preference testing.

Table 1. Formulas for the calculation of yields on:
1. an actual wet basis (AW)
2. a dry weight basis (DW)

1. $\%AW = \frac{\text{Wt. Curd}}{\text{Wt. Milk}}$

2. $\%DW = \frac{\text{\% Total Solids Cheese x Wt. Cheese}}{\text{\% Total Solids Milk x Wt. Milk}} \times 100$

Figure 3. American Dairy Science Association Cheddar Cheese score card presented to experienced judges for preliminary evaluation of Cheddar cheese.

CONTEST

DATE _____ AUSA CHEDDAR CHEESE SCORE CARD U.S.A. _____ CONTESTANT NO.

PERFECT SCORE	CRITICISMS	SAMPLE NO 1	2	3	4	5	6	7	8	9	10	TOTAL GRADE
FLAVOR	CONTESTANT SCORE ➧											
	GRADE SCORE											
	GRADE CRITICISM											
	ACID											
	BITTER											
NO CRITICISM 10	FEED											
	FERMENTED/FRUITY											
	FLAT/LACKS FLAVOR											
	GARLIC/ONION											
	HEATED											
	MOLDY											
NORMAL RANGE 1–10	RANCID											
	SULFIDE											
	UNCLEAN											
	WHEY TAINT											
	YEASTY											
BODY AND TEXTURE	CONTESTANT SCORE ➧											
	GRADE SCORE											
	GRADE CRITICISM											
NO CRITICISM 5	CORKY											
	CRUMBLY											
	CURDY											
	GASSY											
NORMAL RANGE 1–5	MEALY											
	OPEN											
	PASTY											
	SHORT											
	WEAK											
COLOR	ALLOWED PERFECT IN CONTEST ➧	X	X	X	X	X	X	X	X	X	X	
FINISH	ALLOWED PERFECT IN CONTEST	X	X	X	X	X	X	X	X	X	X	
TOTAL	TOTAL SCORE OF EACH SAMPLE ➧											
	TOTAL GRADE PER SAMPLE											

FINAL GRADE _____

RANK _____

TEAM RANK	CODE	GRADE
	1	
	2	
	3	
	TOTAL	
	RANK	

Consumer Panel

Four of six samples of Cheddar cheese from a single trial were presented randomly to each panelist for evaluation of the acceptability and preference. A total of 90 panelists were surveyed for each trial, thus, each cheese was evaluated by 60 panelists (Table 2). A total of 360 responses were obtained from the four trials. Each respondant was required to sign an information and consent form in accordance with the rules of the University Committee on Research Involving Human Subjects.

Acceptability of the cheese was determined from responses to a five-point Hedonic scale consisting of Like, Like Slightly, Neutral, Dislike slightly, and Dislike. Preference of the consumers was determined from rankings of the four cheeses presented to each panelist (Figure 4). Panelists consisted of customers patronizing the MSU dairy store and it was assumed they had prior exposure to this type of product.

Table 2. Consumer Panel - Random number coding and sample distribution pattern.

	1C	1T	3C	3T	5C	5T
Trial #1	847	925	793	285	232	715
Trial #2	213	735	253	827	394	664
Trial #3	367	763	778	606	259	455
Trial #4	899	685	878	691	991	180
1	x	x	x	x		
2	x	x	x		x	
3	x	x	x			x
4	x	x		x	x	
5	x		x			x
6	x	x			x	x
7	x		x	x	x	
8	x		x	x		x
9	x		x		x	x
10	x			x	x	x
11		x	x	x	x	
12		x	x	x		x
13		x	x		x	x
14		x		x	x	x
15			x	x	x	x

60 of each sample prepared and pattern repeated 6 times with samples being presented in different order each time thru pattern. 90 consumers tested on each trial.

Figure 4. Evaluation form presented to each consumer participating in the Cheddar cheese taste panel. Panelists' forms were only identified by consecutive numbers.

Cheddar Cheese Evaluation

Date _____ Panelist _____

1. Taste each sample and place a checkmark ( ) in the box which best describes how you like the cheese.

Sample No.	Like	Like Slightly	Neutral	Dislike Slightly	Dislike
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐
_____	☐	☐	☐	☐	☐

2. List the four samples in order of preference.

Sample No.

_____ most liked

_____

_____

_____ least liked

ANALYTICAL PROCEDURES

Sample Preparation

Samples of milk were taken at the farm with a dipper stored in an iodine sanitizer. The dipper was allowed to drain and then rinsed three times in the milk before the sample was obtained. This same procedure was used when obtaining samples of the milk each day prior to and after pasteurization, and of the whey during and after manufacture of Cheddar cheese. Milk and whey samples were first tested for microbiological quality followed by constituent analyses.

Samples of cheese were obtained after pressing and at 6,12,24,36, and 48 weeks of age. A 5 cm wide cross section of one of the six 4.5 Kg blocks from each batch of cheese was shredded and thoroughly mixed at each of the sampling dates. These samples were used for constituent analysis. A 1 g sample was aseptically removed from the remainder of the block of cheese for microbiological analyses. The remainder of the block was used for organoleptic analysis and removed from further consideration in this study.

All samples of milk, whey and cheese were collected in sterile Whirl-pak bags and stored at 4.4 C or below. Samples for microbiological analyses, titratable acidities,

pH, fat, and total solids were used within 24 hr of sampling. Samples for other analytical procedures were frozen at -20 C until time was available for analysis.

Compositional Analysis

Titratable Acidity

The titratable acidities of milk and whey samples were determined with the Nafis apparatus as described by Atherton and Newlander (1977).

pH

The pH measurements of cheese samples were made with a Chemtrix type 60A digital pH/mv meter equipped with an Orion (model 91-63) pH electrode designed for surface measurements. The pH of milk and whey samples was determined with the same meter but with a Chemtrix combination pH electrode.

Preparation of Whole Casein

The whole casein used as a standard for comparison in the electrophoretic studies was prepared according to a method described by Vanderpoorten and Weckx (1972). Whole milk was obtained from Holstein cows of the Michigan State University dairy herd; the fat was removed by centrifugation for 30 min at 754xg. Final fat content was 0.1% or less. The skimmilk obtained in this way was slowly acidified to pH

4.6 with 1 N HCl. The precipitate was washed three times with distilled water and extracted 5 hr at pH 4.0 in acetic acid so that the milk proteases were removed. The whole, isoelectric casein obtained was washed 3 more times with distilled water, lyophilized and stored at 0 C.

Fat

The fat content of the milk, whey and cheese samples was determined by the Roesse-Gottlieb method with Mojonnier modification (Atherton and Newlander, 1977).

Total Solids

Total solids in milk, whey and cheese were determined by the vacuum oven method (AOAC, 1975).

Salt

Determination of salt in cheese samples was performed by the modified Volhard test described by Kosikowski (1977) with modifications. The washing and decanting for removal of AgCl precipitate was replaced by the addition of 2 ml of acetone to prevent participation of AgCl in the titration.

Free Fatty Acid (FFA Titer)

The rapid silica gel method for measuring FFA in milk (Harper et al., 1956) was adopted for the measurement of FFA titers of the cheeses at all sampling times. Five grams of

cheese were used for analysis and the flow rate of eluant was adjusted to 30 ml/min. Results were expressed as moles FFA/g cheese.

Total Nitrogen

The total nitrogen in milk, whey and cheese was determined by the micro-Kjeldahl method (AOAC, 1975). Sample sizes of 40, 250 and 1200mg were used for cheese, milk and whey, respectively. These required 3-10 ml 0.02 N HCl for titration.

pH 4.6-soluble Nitrogen

Determination of pH 4.6-soluble nitrogen in milk, whey and cheese samples was performed according to a technique based on the method of Sharp (Kosikowski, 1977) with some modifications.

One and one-half grams of cheese were accurately weighed in a 6 oz Whirlpak bag. The cheese was then crushed by rolling a round bottle over the bag. A small amount of extraction solution at 50 C was added and the suspension was mixed thoroughly. This mixture was quantitatively transferred to a 25 ml volumetric flask. The flask was placed in a water bath at 50 C and maintained at this temperature for 1 hr.

The suspension was filtered through a Whatman No. 1 fluted filter paper and 2.0 ml of the 36- and 48-week cheese filtrate and 3.0 ml the 0-24-week cheese filtrate were

placed in 30 ml micro-Kjeldahl flasks. Three milliliters of the whey filtrate and 2.0 ml of the milk filtrate were added to 100 ml micro-Kjeldahl flasks with expansion bulbs in the neck to prevent loss of sample due to foaming. Digestion and distillation steps were conducted as for total nitrogen.

12% TCA-soluble Nitrogen

12% TCA-soluble nitrogen in cheese was determined following the procedure described by O'Keeffe et al. (1976) and Reville and Fox (1978) with modifications.

One and one-half grams of cheese was prepared according to the procedure for pH 4.6-soluble nitrogen. Water at 75 C was used to suspend and transfer the sample to a 25 ml volumetric flask and to fill the flask to 25 ml. Ten grams of milk and whey samples were added directly to the volumetric flasks.

The flask was placed in a water bath at 75 C and maintained at this temperature for 5 min. The suspension was cooled and filtered through a Whatman No. 1 fluted filter paper. To a 10 ml aliquot, 15 ml of 20% TCA solution were added so that the final 25 ml would contain 12% TCA.

A precipitate formed immediately and was removed by centrifugation (Sorvall SuperSpeed centrifuge model RC2-B) at 20,000xg for 30 min at 2 C. Ten milliliters of 0- and 6-week cheese supernatants, 5.0 ml of 12-week or older

cheese supernatants were analyzed for nitrogen using the same types of flasks and procedures as for pH 4.6-soluble nitrogen.

Discontinuous Polyacrylamide Gel Electrophoresis(Disc-PAGE)

Disc-PAGE was run by the method of Ornstein (1964) and Davis (1964) as modified by Melachouris (1969) and adapted for cheese samples.

Sample Preparation

Cheese samples for electrophoretic analyses were prepared following a procedure reported by Ledford et al. (1966). Fifty milligrams of cheese was dissolved in 0.8 ml of distilled water and 2.0 ml of stacking gel buffer containing 7 M urea. The sample solution was warmed to 37 C for 1 hr to effect a layering of fat thus facilitating removal of the aqueous phase for electrophoresis. To demonstrate k-casein, 2 drops of -mercaptoethanol were added and the sample was kept in the water bath an additional 45 min. Immediately before the electrophoretic run, 3 ml of Bromophenol blue solution and 100 ml of saturated sucrose solution were added to the sample. Whole casein was used as a standard for comparison with the cheese samples. A casein sample of 0.01 g was prepared in an identical manner to the above cheese samples. A casein standard was included in

each of the electrophoretic runs. Procedures for the preparation of solutions and electrophoretic gels are included in the Appendix.

Electrophoresis Run

Electrophoresis was conducted in glass tubes with a length of 12.5 cm and an inner diameter of 6 mm. Tubes were placed in a water-cooled Bio-Rad Model 150A electrophoresis apparatus and a constant current of 2 mA/tube was supplied by a Bio-Rad Laboratories Model 500 power supply. Gels were stained in 0.05% (w/v) Coumassie brilliant blue R-250 overnight and destained by diffusion. Detailed procedure for the electrophoresis and destaining are included in the Appendix.

Gel Densitometry

Gels were scanned using a Beckman DU Spectrophotometer, Model 2400 equipped with a gel scanner Model 2520 and a photometer 252 by Gilford Instrument Laboratories, Inc.

This system was augmented by a Hewlett-Packard Integrator Model 3380S. The gels were scanned at a rate of 1.0 cm/min and results recorded at a chart speed of 2.0 cm/min.

Disc-PAGE gels were scanned at a wavelength of 550 nm and attenuation was set at 64. The relative areas of the individual proteins were recorded. The relative mobilities

of the bands were calculated from the total length of the gels and from the distances migrated by individual proteins.

Microbiological Analyses

Standard Plate Counts

Standard Plate Counts (SPC) were performed on samples of raw milk and pasteurized milk, as outlined in Standard Methods for the Examination of Dairy Products (APHA, 1972), to evaluate sanitary practices and pasteurization procedures. Dilutions of the samples in sterile phosphate buffered-distilled (SPBD) water were carried out as in Table 3.

Psychrotrophic Plate Counts

Psychrotrophic Plate Counts (PPC) of raw milk, pasteurized milk, whey, after draining, and cheese, after pressing, were run according to the Standard Methods for the Examination of Dairy Products (APHA, 1972). Milk and whey samples were diluted in SPBD water and cheese samples were prepared and diluted in sterile 2% aqueous sodium citrate. The one gram samples of cheese were macerated in their Whirl-pak bags by rolling with a round bottle. Nine milliliters of sterile 2% sodium citrate were then added to the bag to give the initial 1:10 dilution. The dilutions used for all samples are illustrated in Table 4.

Table 3. Dilutions used to perform SPC's on samples of raw and pasteurized milk.

Sample	Dilutions			
	Control		Experimental	
Raw Milk	1:100	1:1000	1:10,000	1:100,000
Pasteurized Milk	1:10	1:100	1:10	1:100

Table 4. Dilutions used to perform PPC's on samples of raw and pasteurized milk.

Sample	Dilutions			
	Control		Experimental	
Raw Milk	1:10	1:100	1:10	1:100
Pasteurized Milk	1:1	---	1:1	---
Whey	1:10	1:100	1:10	1:100
Cheddar Cheese	1:10	1:100	1:10	1:100

Lactic Bacterial Counts

Lactic Bacterial Counts (LBC) of raw milk, pasteurized milk, whey, and all Cheddar cheeses were used to monitor lactic culture development (Torres, 1979). Preparation of samples and dilution solutions were the same as for PPC and final dilutions may be found in Table 5. The ingredients were dissolved in 1 l of distilled water on a hot plate equipped with a magnetic stirring device. The lactic agar was sterilized at 121 C for 15 min in an autoclave.

Yeast and Mold Count

Yeast and mold (Y+M) counts were run on the cheese after pressing as an indication of possible airborn contamination during the manufacturing step (APHA, 1978). Dilutions were 1:10 in aqueous 2% sodium citrate.

Statistical Analyses

Two statistical designs were used in analyses of variance of the data collected during the various stages of the study. First, a completely randomized, block design, with trials 1 through 4 as blocks, was used to analyze the data from milk, whey and fresh cheese (Gill, 1978). The population was defined by the following model:

$$Y_{ijk} = \mu + T_i + \delta_j + (T\delta)_{ij} + B_k + E_{(ijk)}$$

where: μ = overall mean

Table 5. Dilutions used to perform LBC's on samples of milk, whey and cheeses.

Sample	Dilutions					
	Control			Experimental		
Raw Milk	$1:10^2$	$1:10^3$	---	$1:10^4$	$1:10^5$	---
Pasteurized Milk	$1:10^0$	---	---	$1:10^0$	---	---
Whey	$1:10^5$	---	---	$1:10^5$	---	---
0 Week Cheese	$1:10^6$	$1:10^7$	---	$1:10^6$	$1:10^7$	---
6 Weeks	$1:10^5$	$1:10^6$	---	$1:10^5$	$1:10^6$	---
12 Weeks	$1:10^4$	$1:10^5$	---	$1:10^4$	$1:10^5$	---
24 Weeks	$1:10^3$	$1:10^4$	$1:10^5$	$1:10^3$	$1:10^4$	$1:10^5$
36 Weeks	$1:10^2$	$1:10^3$	$1:10^4$	$1:10^2$	$1:10^3$	$1:10^4$

τ= fixed effects of the ith treatment (t=2)
δ= fixed effects of the jth day of milk storage (d=3)
$\tau\delta$= interaction of the fixed effects of the ith treatment and the jth day of milk storage
B = random effects of the kth block Trial (b=4)
E = residual error

The second design was a split-block design used in conjunction with the data collected during the aging of the cheese (Gill, 1978). The following population model was used for analysis of variance:

$$Y_{ijkl} = \mu + \tau_i + \delta_j + (\tau\delta)_{ij} + B_k + E_{(ijk)} + \nu_l + (\tau\nu)_{il} + (\delta\nu)_{jl} + (\tau\delta\nu)_{ijl} + E_{ijkl}$$

where: $\mu, \tau, \delta, (\delta), B$ were defined as in the above model

E = the sum of the variation due to block interactions
ν = fixed effects of the lth week of aging (a= 5 or 6)
$\tau\nu$ = interactions of the fixed effects of the ith treatment and the lth week of aging
$\delta\nu$ = interactions of the fixed effects of the jth day of milk storage and the lth week of aging
$\tau\delta\nu$ = interactions of the fixed effects of the ith treatment, the jth day of milk storage and the lth week of aging
E = residual error

A general Null hypothesis (H_o) for use with the above models was formulated for statistical analysis.

H_o: There were no statistical differences in the milk, whey or cheese as a result of the fixed effects or their interaction with regard to the following dependant variables:

a) titratable acidity (milk and whey only)
b) pH
c) fat
d) total solids (moisture)
e) total nitrogen
f) pH 4.6-soluble Nitrogen
g) 12% TCA-soluble Nitrogen
h) bacterial counts
i) salt (cheese only)

j) free fatty acid titer (cheese only)
k) electrophoretic pattern (cheese only)
l) sensory quality (cheese only)

H_1 : There were no statistical differences in milk, whey, or cheese as a result of the fixed effects or their interactions with regard to the previously mentioned dependent variables.

Analysis of variance was done on the computer at Michigan State University, using the multiple variance (MANOVA) procedures from the Statistical Package for the Social Sciences (SPSS) (Hull and Nie, 1981).

Curd Firmness

This study was prompted by observations in the original study, therefore, the milk samples were not identical to the ones used earlier. For each of three trials approximately 1600 ml of raw milk was collected from the University farm (trials 1 and 2) or the dairy plant (trial 3). This was split into two 800 ml portions and stored in stoppered glass bottles for 5 days at 4 C. One portion served as a control while the other was inoculated with 0.5% of the bulk lactic starter at the beginning of storage. At the end of the storage period both portions of milk were pasteurized at 63 C for 30 min and cooled to 31 C, then six 100 ml portions of each were placed in 250 ml pyrex beakers for coagulation. The pH of the two portions was also recorded. One percent fresh lactic starter was added to each beaker and incubated at 31 C for one hr at which time 0.7 ml of a 1:40 dilution of a commercial single strength rennet was added to each beaker. These two steps were performed at one minute

grams and multiplied by the acceleration due to gravity to obtain the force applied in cutting the curd. The Instron settings were:

Full Scale	1 kg
Chart Speed	20 cm/min
Crosshead Speed	20 cm/min

Analysis of variance was performed using the following model:

$$Y = \mu + T_i + R_{(i)j} + B_{(ij)k} + E_{(ijk)}$$

where: μ = mean force of cutting curd
T_i = fixed effects of the ith treatment (t=2)
R = random effects of the jth replicate sample nested within the ith treatment
B = random effect of the kth block (trial)
E = residual error

RESULTS AND DISCUSSION

Effects of Storage and Treatment on Milk Quality

Bacteriological Analysis

The mean Standard Plate Counts (SPC) of the control and seeded milk are recorded in Table 6. As expected the seeded milk showed a higher count than the control milk on the 1st day of storage but by the 5th day the control milk had 2.9×10^{7} cfu/ml which was almost double the mean SPC of the seeded milk. The great increase in numbers is due almost entirely to trial I, which had an initial count of 1.6×10^{5} cfu/ml. The standard for grade A farm milk of 1.0×10^{5} cfu/ml (FDA, 1978) was exceeded in trial I and the increase in bacterial counts over the following storage period confirmed the need for starting with a clean milk supply if the product is to be stored for future processing (Gehriger, 1981). Trials II through IV started with a control mean of 5.6×10^{3} cfu/ml and ended with a control mean of 6.2×10^{4} cfu/ml, which placed the milk within grade A standards after 5 days of storage.

The results of the Lactic Bacterial Counts (LBC) confirmed the results of the SPCs. Trial I showed the only increases while trials II through IV showed no significant increases in counts (Table 7). The seeded milk samples were consistently in the 10^{6} -10^{7} cfu/ml range as was expected because starters of optimum growth (10^{8} -10^{9} cfu/ml) were used for inoculation (Jespersen, 1979; Lawrence et al., 1976).

Table 6. Standard Plate Count (SPC) of raw control and seeded milk.

Days of Storage	Treatment	Trials I-IV	Trials II-IV	Trials I
		mean CFU/ml		
1	Control	4.5×10^4	5.6×10^3	1.6×10^5
	Seeded	2.1×10^6	2.7×10^6	2.6×10^5
3	Control	8.4×10^4	1.2×10^4	$>3.0 \times 10^5$
	Seeded	4.5×10^6	3.5×10^6	7.4×10^6
5	Control	2.9×10^7	6.2×10^4	1.2×10^8
	Seeded	1.7×10^7	3.8×10^6	5.8×10^7

Table 7. Lactic Bacteria Count (LBC) of raw control and seeded milk.

Days of Storage	Treatment	Trials I-IV	Trials II-IV	Trials I
		mean CFU/ml		
1	Control	1.4×10^{4}	2.8×10^{3}	4.6×10^{4}
	Seeded	5.8×10^{6}	2.0×10^{6}	5.6×10^{6}
3	Control	1.1×10^{5}	1.1×10^{3}	4.4×10^{5}
	Seeded	4.9×10^{6}	2.6×10^{6}	6.0×10^{6}
5	Control	6.5×10^{6}	3.8×10^{3}	2.6×10^{7}
	Seeded	5.9×10^{6}	2.2×10^{6}	8.4×10^{6}

Juffs and Babel (1975) demonstrated the potential for some commercial lactic cultures to inhibit the growth of psychrotropic bacteria. Psychrotrophic Plate Counts (PPC) of both control and seeded milk in storage indicated no significant differences in the growth of psychrotrophs due to the treatment received. The PPCs of trial I on the 3rd and 5th days of storage were recorded as $>3.0 \times 10^5$ because dilutions were too small, resulting in overcrowded plates (Table 8). The high counts found in SPCs and LBCs for trial I were most likely the result of increased psychrotrophic population. Although it appears the inoculation may have inhibited the growth of bacteria in trial I as represented by lower SPCs and LBCs for the seeded milk, no significance can be placed on those values because they are from a single trial.

The 100 fold increase in mean psychrotrophic count between the 3rd and 5th days of storage indicated at least a 48 hr lag period in the multiplication of psychrotrophic bacteria. A long lag period has been shown to be typical of psychrotrophic growth in milk stored at low temperatures (Cousins, 1982; Thomas and Thomas, 1973). Small decreases in product temperature within the range of 1-8 C have demonstrated significant lengthening of the lag period of psychrotrophic growth (Muir et al., 1978; Richard, 1981). A higher initial contamination or a higher storage temperature might have lead to levels of psychrotrophs that could have demonstrated this lactic culture's inhibitory potential.

Table 8. Psychrotrophic Plate Count (PPC) of raw control and seeded milk.

Days of Storage	Treatment	Trials I-IV	Trials II-IV	Trials I
		mean CFU/ml		
1	Control	1.5×10^4	7.0×10^2	6.1×10^4
	Seeded	1.4×10^4	4.0×10^2	5.4×10^4
3	Control	1.3×10^4	7.9×10^3	$>3.0 \times 10^5$
	Seeded	1.1×10^4	5.6×10^3	$>3.0 \times 10^5$
5	Control	3.3×10^5	3.4×10^5	$>3.0 \times 10^5$
	Seeded	2.2×10^5	1.9×10^5	$>3.0 \times 10^5$

Just prior to manufacture of cheese the milk was pasteurized and samples for the evaluation of the heat treatment's efficiency were taken from the cheese vat before anything was added. Recorded in Table 9 are the results of PPCs, LBCs, and Coliform (Coli) counts. With few exceptions, no colonies could be located on the plates as indicated by values of <30 cfu/ml for PPC and LBC and <10 cfu/ml for Coli. The PPC indicated a lack of thermoduric psychrotrophs such as found by White et al. (1978) and Boyd et al. (1953). The lactic culture was expected to be heat labile (Somkuti et al., 1979), and the LBC of the seeded samples confirmed the destruction of the lactic inoculum. The Coli counts were used as a check for post-pasteurization contamination from equipment and showed that good sanitation practices were observed on all but one day.

Compositional Analyses

The seeding of raw milk with lactic cultures has been shown to cause increased levels of acidity in milk stored at 3.5 C (Juffs and Babel, 1975). Increases of 0.015% to 0.025% in T.A. and decreases of 0.12 to 0.18 in pH were found after 5 days of storage. The results of TA and pH measurements for this experiment were reported in Table 10. The seeding of the milk resulted in a mean difference of 0.005% in TA below the control. The difference between control and seeded milk remained constant over the 5 days of storage. A trend towards increased TA after 5 days storage was found to

Table 9. Bacteria counts from pasteurized milk - control and seeded.

Days of Storage	Treatment	Psychrotrophic Plate Count	Lactic Bacteria Plate Count	Coliforms
		----------CFU/ml----------		
1	Control	<30	<30	<10
	Seeded	<30	<30	<10
3	Control	<30	<30	<10
	Seeded	<30	<30	<10
5	Control	<30	<30	<10
	Seeded	<30	<30	<10

be significant only at the 0.10 level (Figure 5a). Similar changes in acidity were indicated by mean differences in pH of 0.02 to 0.09 units with the seeded milk recording the lower values (Table 10). The 5th day of storage had pH values significantly lower than the 1st and 3rd days of storage (Figure 5b).

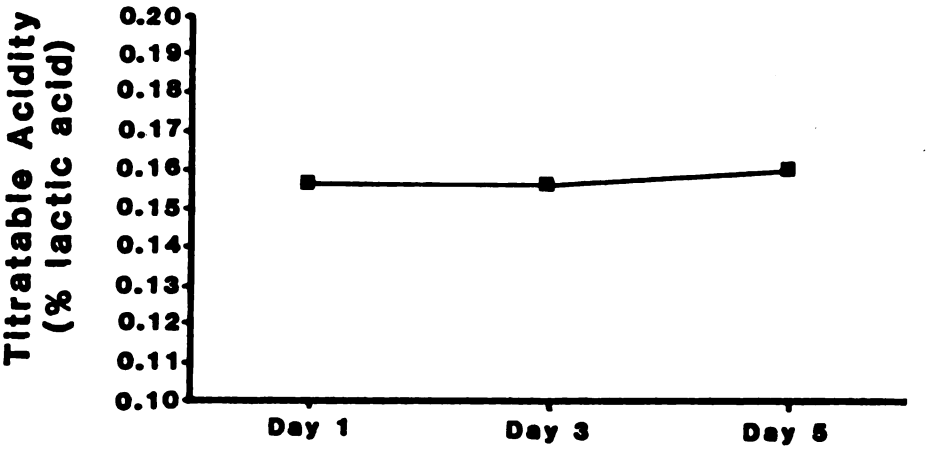
The interaction of treatments and days of storage was not significant with both giving parallel or near parallel plots of factors (Figure 6a & b). Therefore one could not conclude that the acidity of the milk increased at a faster rate in the seeded milk. The lack of higher acid production in the seeded milk was expected because the lactic cultures showed no significant growth as illustrated by the LBC in Table 7.

The increase in acidity was not necessarily detrimental to the milk quality. An increase in acidity is part of the cheese manufacturing process and studies have indicated that increases in acidity, within the range of values found in this study, were clearly linked to increases in the firmness of the rennet curd (Rowland and Soulides, 1942; Cousin and Marth, 1977b). The experience during this study was that the seeded milk did form a firmer clot in the cheese vat, but this could not be objectively measured. Therefore an additional study was performed to confirm or reject the subjective judgments of the researcher.

The results of the curd firmness tests are recorded in Table 11. The difference in mean force required to cut the

Figure 5. The mean titratable acidity (5a) and mean pH (5b) of all milk samples during the five days of storage.

5a.


Titratable Acidity
(% lactic acid)
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
Day 1
Day 3
Day 5

5b.

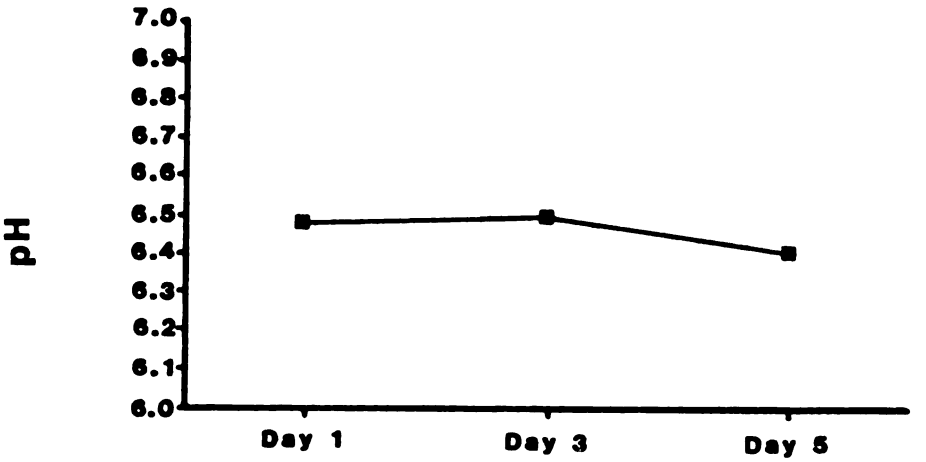
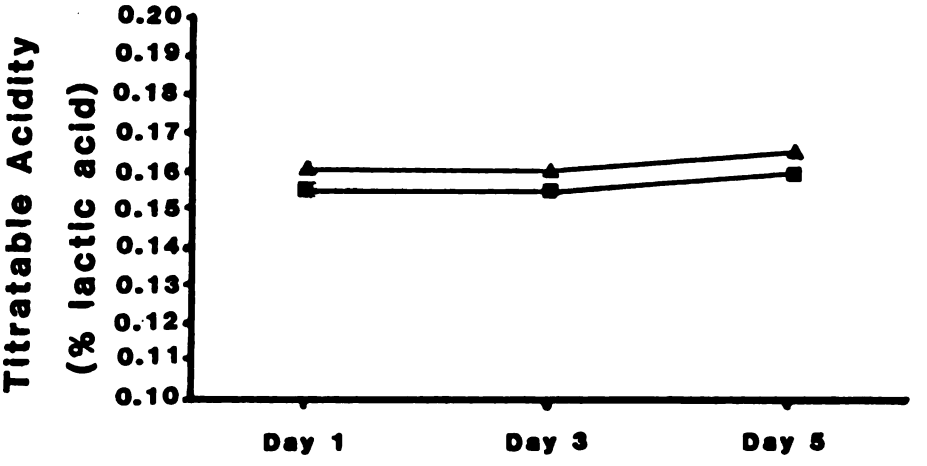

pH
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
Day 1
Day 3
Day 5

Table 10. pH and titratable acidity of control and seeded milk used for the manufacture of Cheddar cheese.

Days of Storage	Treatment	pH	Titratable Acidity
1	Control	6.49	0.155
1	Seeded	6.47	0.160
3	Control	6.52	0.155
3	Seeded	6.47	0.160
5	Control	6.46	0.160
5	Seeded	6.37	0.165

Figure 6. The mean titratable acidity (6a) and mean pH (6b) of the control (■) and seeded (▲) milk during the 5 days of storage.

6a.


Titratable Acidity
(% lactic acid)
0.20
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
Day 1
Day 3
Day 5

6b.

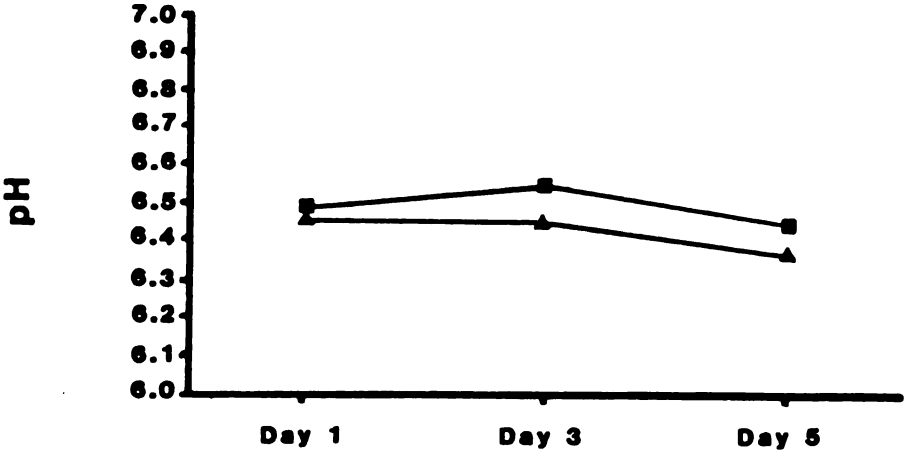

pH
7.0
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
Day 1
Day 3
Day 5

Table 11. pH and rennet curd cutting force after storing control and seeded milk for 5 days.

	Control			Seeded		
Trial	pH	Mean Force (N)	Std. Dev.	pH	Mean Force (N)	Std. Dev.
I	6.59	0.15	0.00	6.11	0.34	0.03
II	6.55	0.14	0.03	6.10	0.24	0.01
III	6.65	0.03	0.01	6.52	0.08	0.00

control and treated curds was significant at <0.001% level when testing the hypothesis that treatment effects equaled zero ($H_o : \tau_i = 0$). Trials I and II had larger pH drops than found in the original study but trial III was very similar to the original data in the magnitude of difference between the control and seeded milks after 5 days of storage. A test of the trial III treatment effects was significant at the 0.005% level. The differences between trials agrees with the work of Bruuen Qvist (Anon., 1981), who demonstrated large variations in curd firmness between different truckloads of milk. The demonstrated increase in firmness of the seeded milk curd may improve the yields of cheese by entrapping more of the soluble solids and reducing the loss through curd fracture during cutting and early stages of cooking.

The fat and total solids contents of the milks demonstrated no statistical differences based on treatment or storage effects. The lack of difference from these factors was expected. The standard deviations ranging from 0.13% to 0.25% and 0.05% to 0.36% for fat and total solids, respectively, were the result of unexpectedly large differences in the milk for the 4 trials (Table 12). The fat content of the milk from the MSU farm ranged from 3.0% to 3.6% fat over a 5 week period. Standardization of the milk to a casein to fat ratio of 0.7:1.0 would have increased yields (Kosikowski, 1977) but the facilities to separate the small amounts of milk used in this study were not

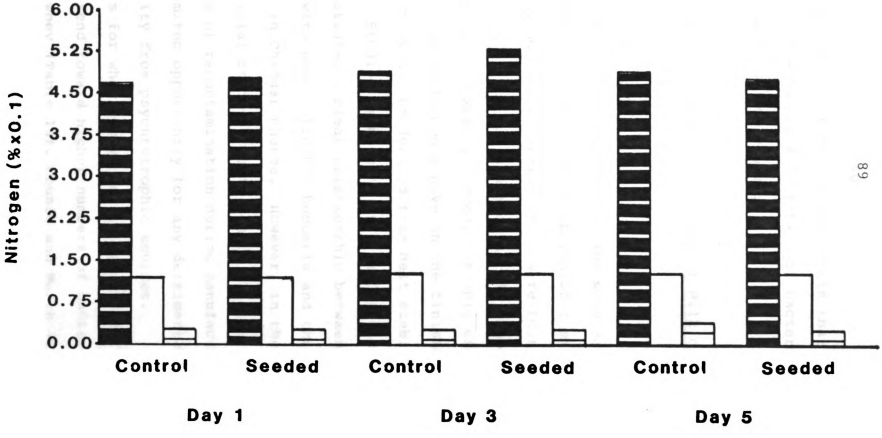
Table 12. Milkfat and total solids content of milk used for Cheddar cheese.

Days of Storage	Treatment	Milkfat	s.d.	Total Solids	s.d.
1	Control	3.39	0.17	11.95	0.24
1	Seeded	3.32	0.20	12.00	0.18
3	Control	3.40	0.22	11.58	0.14
3	Seeded	3.30	0.13	11.47	0.36
5	Control	3.38	0.22	11.57	0.21
5	Seeded	3.33	0.25	11.74	0.05

available. Also the researcher decided not to add skimmilk or cream from another source as that would have added another variable to analysis.

The proteolysis of caseins in milk by psychrotrophic organisms has been well established. Cousins (1976) found that specific activity against the different casein fractions was dependent on the species of bacteria contaminating the milk supply. Pseudomonas spp., the most commonly isolated psychrotrophs, have demonstrated the ability to hydrolyze both α_s - and β- casein (Cousin, 1976; Driessen, 1981). In Finland, milk stored at 4 C for 4 days showed a 5.5% reduction in the ratio of casein nitrogen to total nitrogen (Antila, 1982). A common factor in all of the above mentioned studies was the increase of psychrotrophic bacterial counts to at least 10^6 cfu/ml. The mean PPCs for this study were 3.3×10^5 cfu/ml and 2.2×10^5 cfu/ml for control and seeded milks after 5 days storage (Table 8). The lactic bacteria in the seeded milk showed no significant growth during the storage period. Therefore, one would not expect to see detectable differences in the nitrogen fractions due to bacterial proteolytic activity in this study. In figure 7 the levels of total non-casein (pH 4.6 soluble) and non-protein (12% TCA soluble) nitrogen have been graphically illustrated. The total nitrogen was expected to be constant and did not show any statistical variation. The lack of change in the non-casein and

Figure 7. The total (▬), non-casein (▭) and non-protein (▤) nitrogen content of control and seeded milk during 5 days of storage.


Nitrogen (%xO.1)
6.00
5.25
4.50
3.75
3.00
2.25
1.50
0.75
0.00
Control
Seeded
Control
Seeded
Control
Seeded
Day 1
Day 3
Day 5

non-protein nitrogen confirm the hypothesis that no changes would be detectable during 4 C storage if bacteria counts were kept below below 10^6 cfu/ml.

Effects of Storage and Treatment of Milk on Whey

Bacteriological Analyses

The results of PPCs and LBCs of the whey samples are recorded in Table 13. With the exception of 1 sample out of a total of 24, no colony forming units were found on PPC plates. Therefore, one could state that if proper sanitation procedures were followed, the only effects that psychrotrophic bacteria could have on the finished products and by-products would be derived from heat stable proteases and lipases. Studies by Cousin (1976) and Law et al. (1976) demonstrated a clear relationship between milk precultured with psychrotrophic bacteria and the development of rancidity in Cheddar cheese. However, in the present study the initial psychrotrophic counts of milk combined with the lack of recontamination during manufacture indicated limited opportunity for any detrimental effects on product quality from psychrotrophic sources.

The LBCs for whey showed no statistical differences but did show a trend toward higher numbers of bacteria in the seeded milk whey (Table 13). Cousin and Marth (1976) found that lactic acid was produced at a higher rate in milk precultured with psychrotrophic bacteria. They proposed

Table 13. Bacteria counts for whey from control and seeded milk used for the manufacture of Cheddar cheese.

Days of Milk Storage	Milk Treatment	Psychrotrophic Plate Count	Lactic Bacteria Plate Count
		----------CFU/ml----------	
1	Control	<30	1.91×10^7
	Seeded	<30	4.49×10^7
3	Control	<30	1.04×10^7
	Seeded	<30	1.93×10^7
5	Control	<30	0.91×10^7
	Seeded	<30	1.79×10^7

that the proteolytic activity of the psychrotrophs modified milk proteins releasing nitrogen fractions more usable by the starter culture. A possible explanation for the trend in this study might be that during the inoculation of the starter for seeding a similar proteolytic modification was occurring. Therefore, the seeding of the milk included not only the lactic culture but also an improved media for the growth of more culture during manufacture.

Compositional Analyses

The pH and T.A. of the whey were recorded at the time of milling and means are presented in Table 14. Milling was started when whey drippings attained a T.A. of 0.45% to provide a cheese close to the maximum allowable moisture content (Wilster, 1969; Wilson and Reinbold, 1965). The mean titratable acidities ranged from 0.47 to 0.50% and the mean pH ranged from 5.21 to 5.43. Variations from the target T.A. of .45% were likely due to error in sampling procedures. Volumes of whey necessary to measure the T.A. and the pH were difficult to obtain due to the small amount of curd cheddaring in the vat.

The mean fat and total solids content are also recorded in Table 14. These values showed no statistical differences and were well within the ranges of 0.20 - 0.35% fat and 6.50 to 7.00% total solids found in the literature (Wilster, 1969; Kosikowski, 1977). The observed increase in curd firmness of the seeded milk had no statistically significant

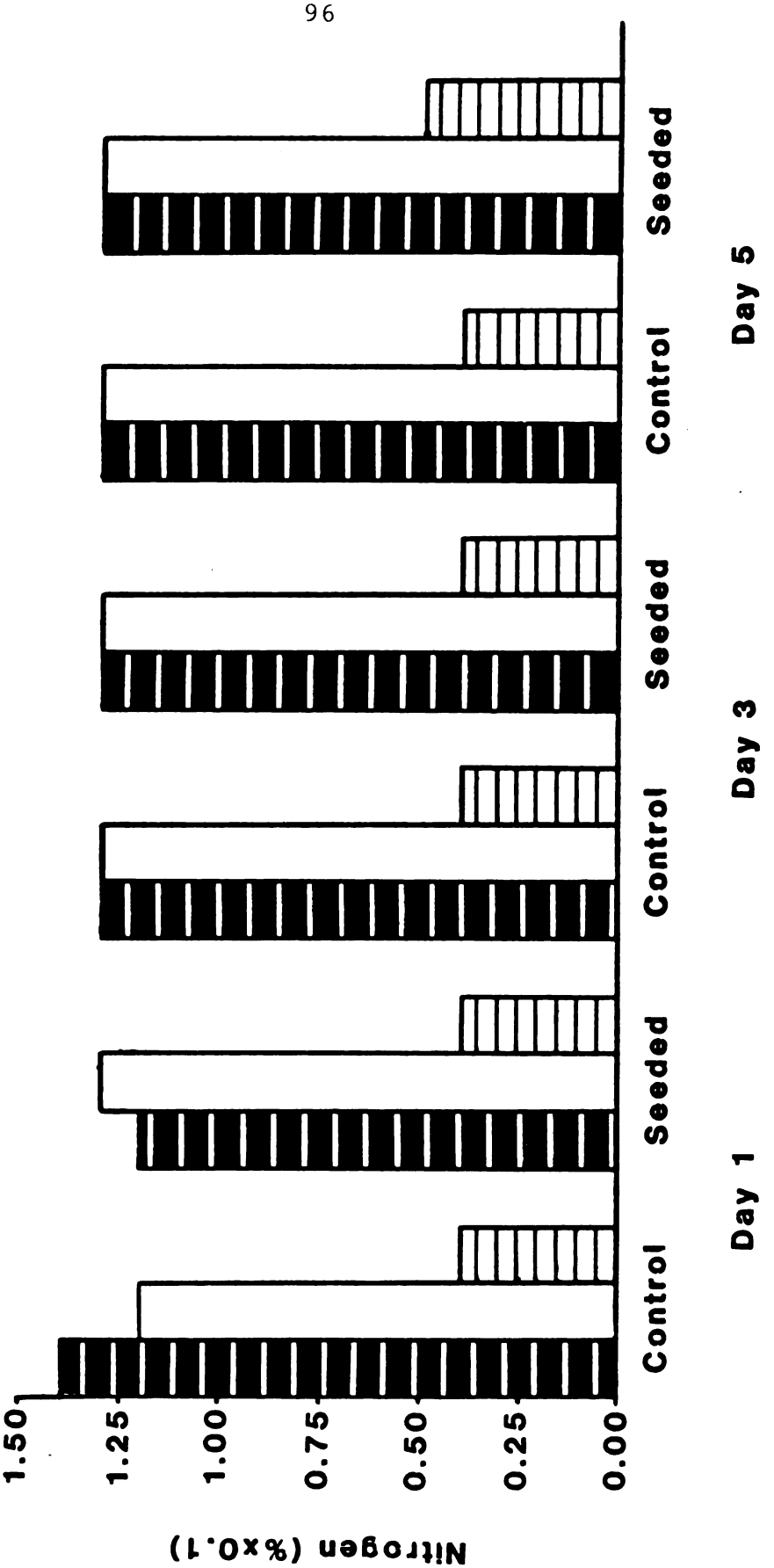
Table 14. Percent composition of whey from control and seeded milk used for the manufacture of Cheddar cheese.

Days of Milk Storage	Milk Treatment	pH	Titratable Acidity	Fat	Total Solids
		----------%			----------
1	Control	5.21	0.50	0.26	6.83
	Seeded	5.25	0.49	0.21	6.81
3	Control	5.40	0.49	0.29	6.73
	Seeded	5.43	0.49	0.22	6.57
5	Control	5.31	0.47	0.22	6.70
	Seeded	5.30	0.47	0.22	6.73

effect on the retention of fat or total solids in the cheese. However, it was noted that the opportunity for loss of small amounts of whey was significant in the small cheese vats making statistical evaluations difficult. A small loss of whey could have made a significant difference in this study while the same size loss in a large vat (2500 Kg) might not be significant to compositional analyses.

Total, non-casein and non-protein nitrogen (Figure 8) showed no statistical differences with regards to treatment or storage. There were also no significant differences between the percentages of total and non-casein nitrogen as would be expected if a good rennet clot was formed.

Figure 8. The total (▬), non-casein (□) and non-protein (⊟) nitrogen content of whey from control and seeded milk used for the manufacture of Cheddar cheese.


Nitrogen (%x0.1)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Control
Seeded
Control
Seeded
Control
Seeded
Control
Seeded
Day 1
Day 3
Day 5

Effects of Treatment and Storage of Milk on Cheddar Cheese

Compositional Analysis

The mean pH value of the cheese after removal from the press ranged from 5.10 to 5.29 (Table 15). The means were not significantly different and were within the normal range of pH for Cheddar cheese after 24 hr (Wilster, 1969). Studies have demonstrated that values for pH above this range have resulted in rancid, fermented or oxidized defects while pH values below have resulted in unclean, sulphide, bitter and sour defects in cheese flavor (Kristofferson, 1967; Wilson et al., 1945).

The mean fat content of the milk used in this study was about 3.4% which should have given a fat content in cheese of about 33.6% in cheese of 37% moisture (Wilson and Reinbold, 1965). The mean fat content of the cheese ranged from 30.62% to 31.50% (Table 15). The reason for this apparently low fat content in the cheese was explained by the moisture content which ranged from 39.46% to 40.93% (Table 15). If the moisture were lower the fat content would have been proportionately higher resulting in near normal levels of fat. Food and Drug Administration standards of identity for Cheddar cheese state that moisture should have been less than 39% (FDA, 1968). High moisture cheese has been attributed to 1) improper standardization of milk, 2) knives with too much space between wires, 3) insufficient

Table 15. Composition of Cheddar cheese manufactured from control and seeded milk.

Days of Milk Storage	Milk Treatment	pH	Fat		Moisture		Salt	
			%					
			mean	s.d.	mean	s.d.	mean	s.d.
1	Control	5.13	31.50	1.29	39.67	1.67	1.19	0.03
	Seeded	5.10	30.87	1.18	39.46	1.75	1.23	0.12
3	Control	5.28	31.32	1.31	40.02	0.94	1.21	0.06
	Seeded	5.29	30.95	0.90	40.93	0.78	1.23	0.03
5	Control	5.24	31.25	0.50	39.98	0.77	1.28	0.15
	Seeded	5.18	30.62	1.11	40.61	0.48	1.20	0.18

cooking time, 4) too few turnings of blocks during cheddaring, 5) too much stacking during cheddaring, and 6) not enough salt (Kosikowski, 1977). A lack of standardization, a couple of missing knife wires and rapid acid development causing shortened cooking times contributed to the moisture problems in this study.

A significantly greater percent fat was found in the control milk cheese and was most likely the result of a trend to slightly lower levels of moisture. The increase in curd firmness, discussed previously, may have resulted in a trend towards increased retention of moisture in the seeded milk cheese.

Salt levels ranging from a mean of 1.19% to 1.28% (Table 15) may also have resulted from the high moisture content. Cheddar cheese normally contains between 1.6% and 1.9% salt (Wilson and Reinbold, 1965).

Several different compositional ratios have been used to assess the quality or potential quality of Cheddar cheese. The Fat:Dry Matter (FDM) ratio has been set at >50% in the Federal Standards of Identification (FDA, 1968). The manufacture of a cheese with a FDM ratio significantly higher than that necessary to safely meet the minimum standard would not increase the acceptability of the cheese to the consumer (Wilson and Reinbold, 1965). The means of FDM ratios ranged from 50.99% to 52.42% (Table 16) and statistical analysis showed no significant differences.

Table 16. Compositional ratios of Cheddar cheese manufactured from control and seeded milk.

Days of Milk Storage	Milk Treatment	Fat:Dry Matter (FDM)	Moisture:Nonfat Substance (MNFS)	Salt:Moisture (S/M)
		----------%	----------	
1	Control	52.20	57.89	3.00
	Seeded	50.99	57.07	3.12
3	Control	52.42	58.19	3.01
	Seeded	52.37	59.27	3.00
5	Control	52.07	58.16	3.21
	Seeded	51.56	58.55	2.96

Therefore, all of the cheeses met both the legal standard and the consumer acceptability standard for fat content in dry matter.

Lawrence and Gilles (1980) advocated the use of the Moisture:Non-Fatty Substance (MNFS) ratio because moisture has a greater effect per unit change on ripening than fat. They used the MNFS ratio to predict the quality of young cheese and found the normal range to be 53-57%. Mean ratios of 57.07-59.27% MNFS (Table 16) indicated that the moisture level of the experimental cheese was too high and ripening should have proceeded at a rate faster than normal. The cheese in the study would not have been expected to be of high quality at one year of aging.

Another useful ratio proposed by Gilles and Lawrence (1973) was the Salt:Moisture (S:M) ratio, which should be 4-6% for high quality cheese. A range of S:M ratio means of 2.96-3.21% (Table 16) indicated that the experimental cheeses lacked the proper amount of salt necessary for proper aging. Low salt levels and high moisture levels combined indicated a potential for rapid ripening of the cheese and possible defects upon extended aging.

The results of analyses for nitrogen and free fatty acids (FFA) are recorded in Table 17. Total nitrogen (TN) was the only factor to show significant differences as a result of treatment. The control cheese was higher in TN than the seeded milk cheese. The differences were likely due to increased moisture retention in the seeded milk

Table 17. Nitrogen content and free fatty acid titer of Cheddar cheese manufactured from control and seeded milk.

Days of Milk Storage	Milk Treatment	Total Nitrogen	pH 4.6 Soluble Nitrogen	12% TCA Soluble Nitrogen	Free Fatty Acid Titer
		----------%	----------		umol FFA/g
1	Control	3.71	0.32	0.17	9.05
	Seeded	3.68	0.30	0.17	8.69
3	Control	3.70	0.28	0.15	8.57
	Seeded	3.63	0.31	0.18	8.89
5	Control	3.69	0.30	0.14	8.51
	Seeded	3.61	0.33	0.15	8.67

cheese and paralleled the differences in fat content. Total nitrogen ranged from 3.61-3.71% and pH 4.6-soluble (non-casein) nitrogen (NCN) accounted for less than 10% of the TN with a range of 0.28-0.33%. Non-protein nitrogen (NPN), as represented by the 12% TCA-soluble fraction, was about 50% of the NCN. The small amounts of NCN and NPN could have been explained by the entrapment of whey in the casein matrix.

Law et al. (1976) found less than 10 µmol FFA/g in cheeses manufactured from milk with and without psychrotrophic contamination during storage. Therefore, the range of 8.51-9.05 µmol FFA/g cheese indicated normal levels of FFA at the end of pressing. Studies have shown that rancidity in cheese caused by psychrotrophic lipases was not apparent until 3-6 months of aging (Cousins, 1976; Law et al., 1976).

Cheese Yield and Solids Recovery

The predicted actual yield of 40% moisture Cheddar cheese from milk testing 3.5% fat would be 9.92% (Wilson and Reinbold, 1965). Given that the milk in this experiment had a mean fat content of about 3.4% fat, the range of 9.65-9.88% actual yield would be within expected limits (Table 18). Yield on a dry weight basis ranged from 48.73-50.20% (Table 18). Solids recovery is reported in Table 18 and ranged from 97.14-99.10%.

Table 18. Yields and solids recovery of Cheddar cheese manufactured from control and seeded milk.

Days of Milk Storage	Milk Treatment	Actual*	Dry Weight**	Solids Recovery***
		----------%		----------
1	Control	9.65	48.73	97.65
	Seeded	9.74	49.16	97.14
3	Control	9.75	50.20	98.16
	Seeded	9.74	50.19	99.10
5	Control	9.66	50.11	98.62
	Seeded	9.88	49.94	97.70

* Actual Yield = $\frac{\text{Wt. Cheese}}{\text{Wt. Milk}} \times 100$

** Dry Weight = $\frac{\text{\% Total Solids Cheese x Wt. Cheese}}{\text{\% Total Solids Milk x Wt. Milk}} \times 100$

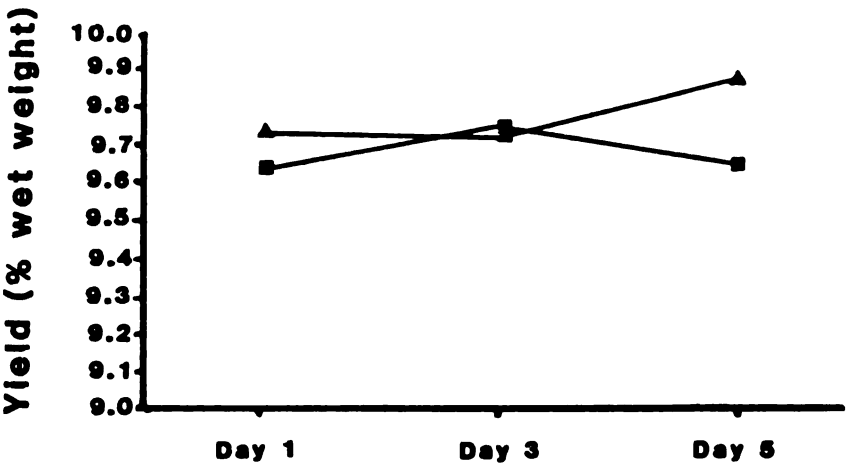
*** Solids Recovery =

$$\frac{(\text{\% TS Cheese x Wt. Cheese}) + (\text{\%TS Whey x Wt. Whey})}{(\text{\%TS Milk x Wt. Milk}) + (\text{\%TS Starter x Wt.}) + (\text{Wt. Salt})} \times 100$$

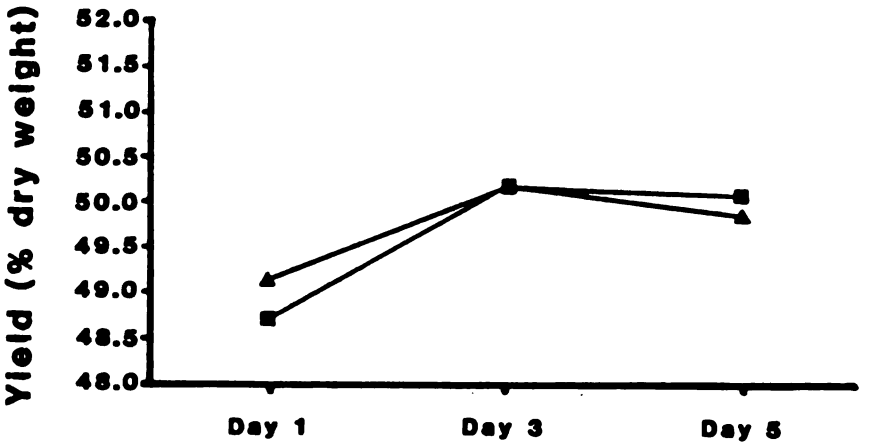
No statistically significant differences were found in the yields or solids recovery. Graphic illustrations of the data indicated a trend toward higher yields and recoveries on the third and fifth days (Figure 9). The lower pH could have improved yields through firmer curd formation but no correlation was found between those factors. Ali et al. (1980a) demonstrated that decreasing yields from milk stored for up to 48 hr were paralleled by increases in soluble-phase casein. However, after 72 hr of storage the yields were better than at 48 hr and the soluble-phase casein had decreased. The milk used in the present study was 6-48 hr old when used for the first day cheeses, but was 54-96 hr old when the third day cheese was made. Ledford et al. (1968) showed that soluble-phase caseins are more susceptible to proteolysis by rennin than micellar caseins. There may have been more soluble-phase casein in the milk on the 1st day than on the 3rd or 5th days but the analytical techniques used were unable to assess this variable. A factor that may have prevented statistical significance from appearing in the culture study would have been the small size of the batches. A large volume is required to avoid large errors caused by small sampling or spillage errors (Sellars, 1982).

Figure 9. The mean actual (9a) and dry weight (9b) yields and mean solids recovery (9c) of Cheddar cheese manufactured from control (■) and seeded (▲) milk stored for 1,3 and 5 days.

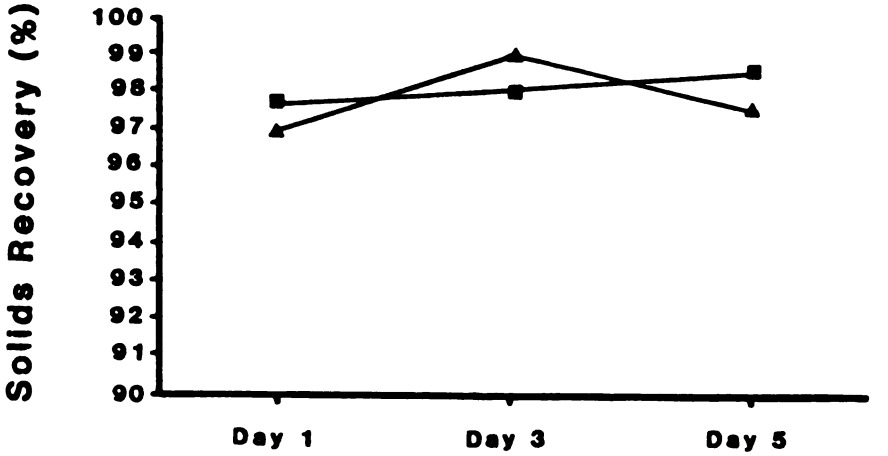
9a.


Yield (% wet weight)
10.0
9.9
9.8
9.7
9.6
9.5
9.4
9.3
9.2
9.1
9.0
Day 1
Day 3
Day 5

9b.


Yield (% dry weight)
52.0
51.5
51.0
50.5
50.0
49.5
49.0
48.5
48.0
Day 1
Day 3
Day 5

9c.

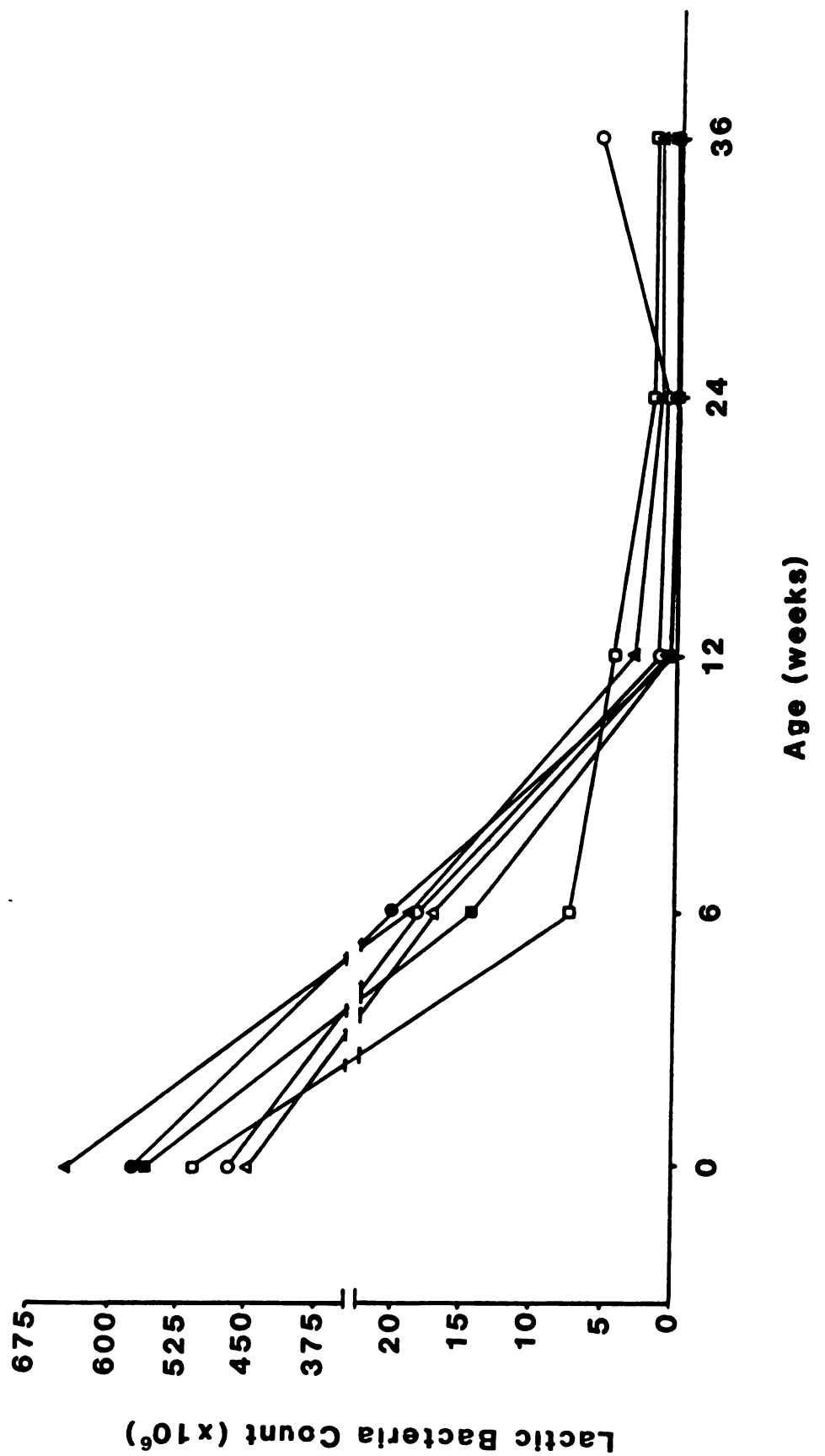

Solids Recovery (%)
100
99
98
97
96
95
94
93
92
91
90
Day 1
Day 3
Day 5

Effects of Treatment and Storage of Milk on Cheese During Ripening

Bacteriological Analysis

The maximum cell density of Str. cremoris during cheese manufacture has been shown to occur at the time of milling by Dawson and Faegan (1957). They demonstrated that cell density declined rapidly during the first 4-8 weeks of ripening, leaving few if any viable cells. Lactic bacterial counts on the cheese from the present study were in agreement with previous work showing approximately 99% decreases in viable cell density by the 12th week of ripening (Figure 10). The viable cell density showed slower decline from 12 weeks to 36 weeks indicating the possible presence of bacteria other than the starter culture. In his review of the bacteriology of cheese flavor, Mabbit (1961) indicated that lactobacilli are commonly found in aged cheese and may increase from 10 cfu/ml in fresh cheese to 10^6 cfu/ml in 2-4 month old cheese. Other research has also indicated that maximum numbers of lactobacilli are present at 3-6 months of ripening at which point they are the predominant flora in the cheese (Johns and Cole, 1959). Therefore, the bacteria that showed the slow rate of decline from 12 weeks to 36 weeks may have been lactobacilli rather than starter streptococci. Yeast and mold counts indicated

Figure 10. The lactic bacteria count of Cheddar cheese manufactured from control and seeded milk stored for 1,3 and 5 days and ripened for 0,6,12,24 and 36 weeks. Day 1 - Control (▫) and Seeded (▵), Day 3 - Control (○) and Seeded (■) and Day 5 - Control (▲) and Seeded (●).


Lactic Bacteria Count ($x10^6$)
675
600
525
450
375
20
15
10
5
0
0
6
12
24
36
Age (weeks)

little or no contamination of the cheese by these types of organisms during the manufacturing or ripening (data not given).

Compositional Analyses

pH vs. Age

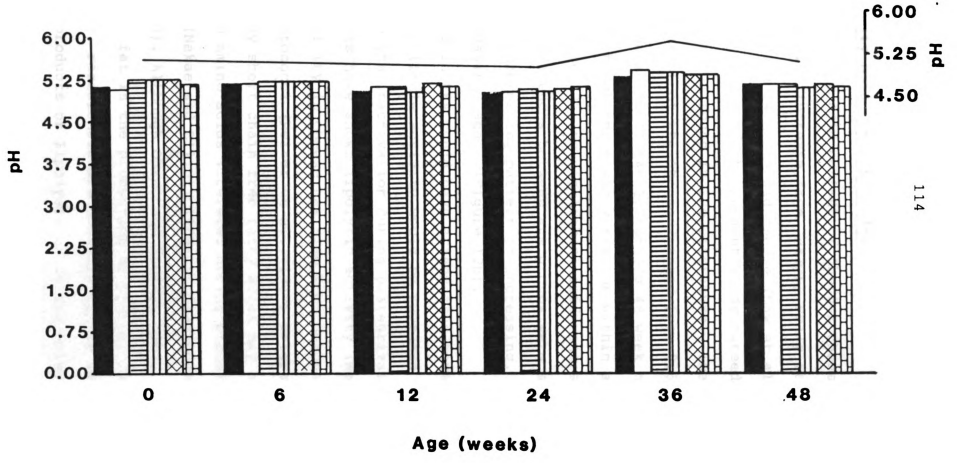
Within a few days of milling, the pH of Cheddar cheese should be 5.05-5.20 and for the following months should increase at an increasing rate until at the end of one year the pH has reached 5.30 to 5.50 (Wilster, 1969; Wong, 1979; Kosikowski, 1977). This increased pH is due largely to the degradation of lactic acid to nonacidic by-products and less highly dissociated acids, such as, acetic and carbonic acids, and the accumulation of alkaline metabolites from protein degradation (Wong, 1974). Mean pH values for control and seeded milk cheeses were recorded in Table 19 and graphically represented in Figure 11.

No significant differences were found in pH, based on the effects of seeding or storage of milk. This relationship was illustrated by the relative equivalence of bar height within each age group (Figure 11). The pH values did show a significant difference with regard to ripening period. A slow decrease was noted for 24 weeks followed by a rapid increase at week 36. The increase was followed by significant decrease during this last 12 weeks of ripening.

Table 19. pH of control and seeded milk cheese during 48 weeks of aging at 4.4 C.

Days of Milk Storage	Milk Treatment	Aging Time (Weeks)					
		0	6	12	24	36	48
1	Control	5.13	5.18	5.02	5.00	5.32	5.13
	Seeded	5.10	5.17	5.11	5.04	5.43	5.11
3	Control	5.28	5.19	5.11	5.09	5.40	5.16
	Seeded	5.29	5.20	5.04	5.07	5.38	5.12
5	Control	5.24	5.22	5.13	5.10	5.33	5.16
	Seeded	5.18	5.20	5.12	5.14	5.32	5.14

Figure 11. The mean pH of control and seeded milk cheese during 48 weeks of ripening at 4.4 C. Cheese from control milk stored for 1 (■), 3 (▤) and 5 (▩) days and seeded milk stored for 1 (□), 3 (▥) and 5 (▦) days.


6.00
5.25
4.50
pH
6.00
5.25
4.50
3.75
3.00
2.25
1.50
0.75
0.00
pH
0
6
12
24
36
48
Age (weeks)

Free Fatty Acid Titer vs. Age

The free fatty acid content of Cheddar cheese has been shown to gradually increase from 7-8 µmol FFA/g fresh cheese to 14-16 µmol FFA/g 14 month old cheese (Law et al., 1976a,b). The results of the present study agreed with the magnitude of values found in the literature. The mean free fatty acid content of the cheeses ranged from 8.51-9.05 µmol/g fresh cheese to 11.64-13.48 µmol/g 48 week old cheese (Table 20). Although there was variation within age groups as seen in Figure 12a, no significant differences were found. The differences between age groups were significant and showed an interesting pattern of increasing, decreasing and then increasing, again (Figure 12b).

Increases in FFA could have arisen from several sources. Very little activity would be expected from milk lipase due to its inactivation during pasteurization. Rennet extracts have shown lipolytic activity (Wong, 1974). Lipolytic activity in cheese has also been demonstrated by starter streptococci (Reiter et al., 1967; Peterson et al., 1948a,b). Many short chain free fatty acids may also be produced from amino acids liberated during protein degradation (Nakae and Elliot, 1965a,b; Stadhouders and Veringa, 1973). Although Ohren and Tuckey, 1969, showed the importance of fat in the production of typical Cheddar cheese flavor, researchers are not sure if the importance lies in the products of lipolysis or in the solvent properties of the fat.

Table 20. Free fatty acid (FFA) titer of control and seeded milk cheese during 48 weeks of storage.

Days of Milk Storage	Milk Treatment	Aging Time (Weeks)					
		0	6	12	24	36	48
		----------μ mol FFA/g cheese----------					
1	Control	9.05	9.37	10.73	11.38	11.09	13.48
	Seeded	8.69	9.18	10.37	12.27	10.30	13.42
3	Control	8.57	9.11	11.13	10.89	10.32	13.13
	Seeded	8.89	8.77	10.56	10.93	10.92	12.40
5	Control	8.51	8.93	9.83	10.46	10.43	12.66
	Seeded	8.67	9.26	10.52	10.73	10.26	11.64

Figure 12a. The mean free fatty acid titer of control and seeded milk cheese during 48 weeks of ripening at 4.4 C. Cheese from control milk stored for 1 (■), 3 (▤) and 5 (▩) days and seeded milk stored for 1 (□), 3 (▥) and 5 (▦) days.

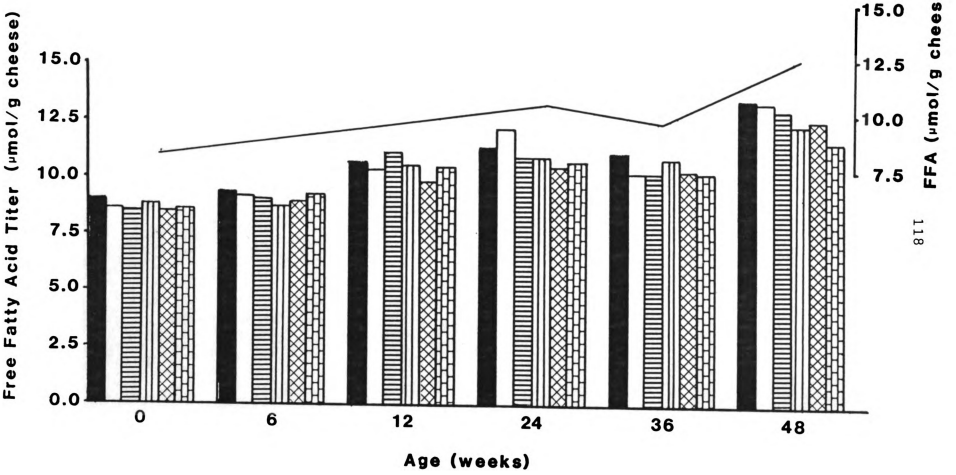
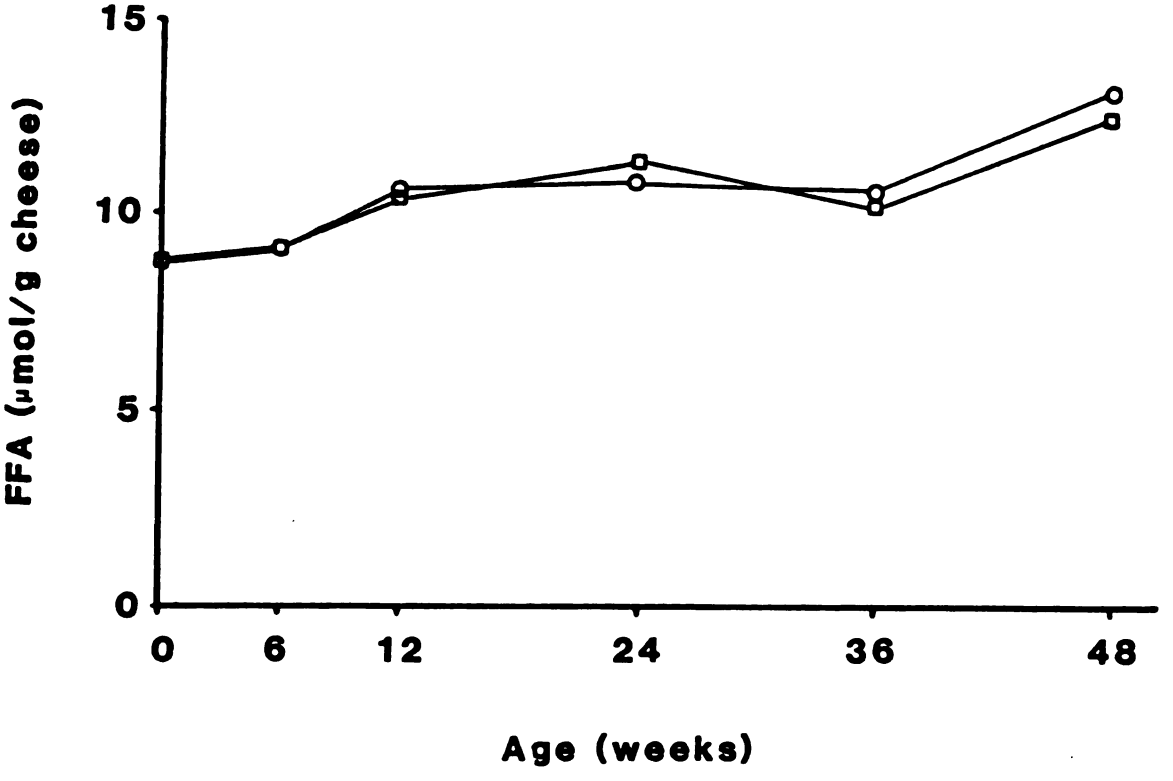

Free Fatty Acid Titer (µmol/g cheese)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0
6
12
24
36
48
Age (weeks)
FFA (µmol/g cheese)
15.0
12.5
10.0
7.5

Figure 12b. The effects of ripening on the free fatty acid content of Cheddar cheese manufactured from control (o) and seeded (◘) milk stored for 1,3 and 5 days.


15
10
5
0
FFA (µmol/g cheese)
0
6
12
24
36
48
Age (weeks)

The decrease in FFA between 24 and 36 weeks of ripening was unexpected, as was the decrease in pH after 36 weeks. During the first 24 weeks of ripening the increase in FFA content and microenvironmental changes may have shifted the equilibrium with ethanol towards the formation of ethyl esters (Hart and Schuetz, 1978). The formation of ethanol and ethyl esters has been demonstrated in a cheese system by several researchers (Law and Sharpe, 1977; Bills et al., 1965). Lowered FFA contents and increases in basic protein degradation products may account for the increase in pH between 24 and 36 weeks. Assuming that only limited amounts of ethanol would be available for esterification, one might expect a subsequent increase in FFA content. The increase may have been intensified by the liberation of intracellular enzymes of lactobacilli. Peterson and Johnson (1945) showed that lactobacilli showed no lipolytic activity until autolysis after maximum cell densities had been attained. If lysis of the lactobacilli did not take place until at least 24 weeks and allowance was given for the diffusion of the lipases from localized colonies to the fat substrate, the increase in FFA and decrease in pH after the 36 week ripening period may have been due to lipase activity higher than in the younger cheeses.

Nitrogen Distribution vs. Age

The non-casein nitrogen (NCN) content of both the control and seeded milk cheeses showed increases of just

over 300% at 48 weeks of ripening. The mean NCN content range was 0.28-0.33% at 0 time and increased to a range of 0.95-1.08% at 48 weeks (Table 21). The increase was rapid during the first 6 weeks followed by a decrease in the rate of NCN accumulation (Figure 13a). Figure 13b illustrates the significantly higher levels of NCN in seeded milk cheese.

Non-protein nitrogen (NPN) accounted for 45-58% of NCN in fresh cheese and showed a slight increase, i.e. 56-67% of NCN, at 48 weeks. Mean percentages for NPN increased from 0.14-0.18% to 0.58-0.68% during the 48 weeks of ripening (Table 22). Rapid increases in NPN for the first 6 weeks followed by a slower rate of increase paralleled the pattern of NCN increases (Figures 14a,b). No significant differences resulted from storage or seeding of the milk. The difference in NCN between control and seeded milk cheeses must be due to larger amounts of non-casein protein components in the seeded milk cheeses.

The ability of decarboxylase produced by lactic streptococci to remain active after heating to 80 C for 60 min has been demonstrated by Oliver (1952). Additionally, the activity of proteases and lipases of psychrotrophs after severe heat treatment has been well established (Adams et al., 1975; Barach et al., 1976; Richardson, 1981; Cousin, 1976; Law et al., 1976a). Therefore, one could hypothesize that some of the cell wall-bound extracellular proteinases of lactic streptococci survived pasteurization. Several studies have demonstrated the ability of these streptococcal

Table 21. Non-casein nitrogen content of control and seeded milk cheese during 48 weeks of aging.

Days of Milk Storage	Milk Treatment	Aging Time (Weeks)					
		0	6	12	24	36	48
		% NCN					
1	Control	0.32	0.62	0.73	0.89	1.08	1.08
	Seeded	0.30	0.63	0.77	0.88	1.04	1.11
3	Control	0.28	0.59	0.71	0.83	0.92	0.98
	Seeded	0.31	0.64	0.74	0.91	1.01	1.08
5	Control	0.30	0.61	0.70	0.85	0.93	0.95
	Seeded	0.33	0.67	0.79	0.91	1.04	1.01

Figure 13a. The mean non-casein nitrogen content of control and seeded milk cheese during 48 weeks of ripening at 4.4 C. Cheese from control milk stored for 1 (■), 3 (▤) and 5 (▩) days and seeded milk stored for 1 (□), 3 (▥) and 5 (▦) days.

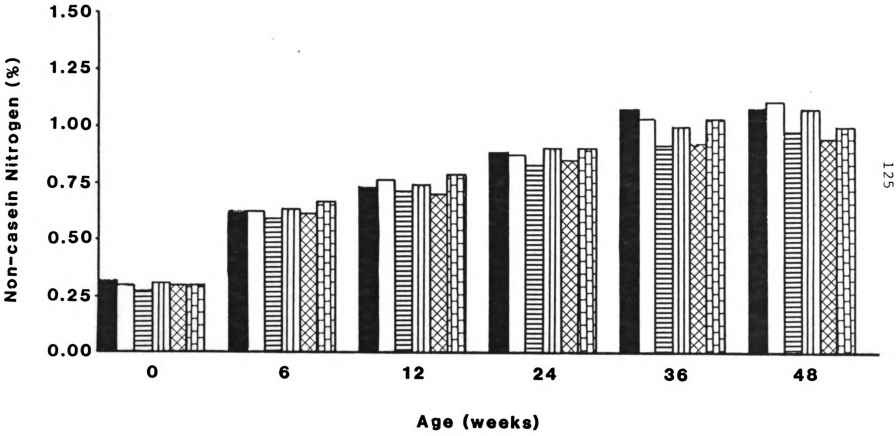

Non-casein Nitrogen (%)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
6
12
24
36
48
Age (weeks)

Figure 13b. The effects of ripening on the non-casein nitrogen content of Cheddar cheese manufactured from control (o) and seeded (o) milk stored for 1,3 and 5 days.

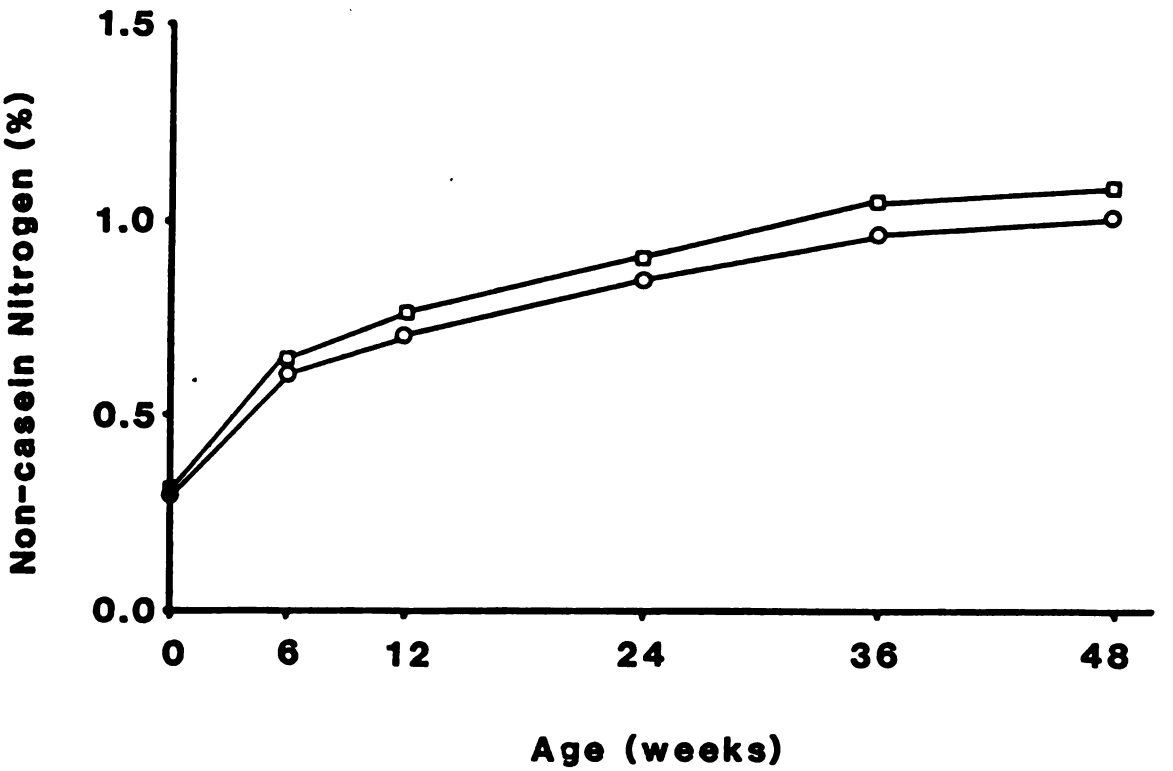

1.5
1.0
0.5
0.0
Non-casein Nitrogen (%)
0
6
12
24
36
48
Age (weeks)

Table 22. Non-protein nitrogen content of control and seeded milk cheeses during 48 weeks of aging.

Days of Milk Storage	Milk Treatment	Aging Time (Weeks) 0	6	12	24	36	48
		% NPN					
1	Control	0.17	0.30	0.45	0.55	0.60	0.68
	Seeded	0.17	0.32	0.45	0.50	0.60	0.66
3	Control	0.15	0.31	0.41	0.43	0.52	0.62
	Seeded	0.18	0.32	0.42	0.53	0.52	0.61
5	Control	0.14	0.33	0.39	0.41	0.56	0.64
	Seeded	0.15	0.33	0.39	0.44	0.52	0.58

Figure 14a. The mean non-protein nitrogen content of control seeded milk cheese during 48 weeks of ripening at 4.4 C. Cheese from control milk stored for 1 (■), 3 (▤) and 5 (▩) days and seeded milk stored for 1 (□), 3 (▥) and 5 (▦) days.

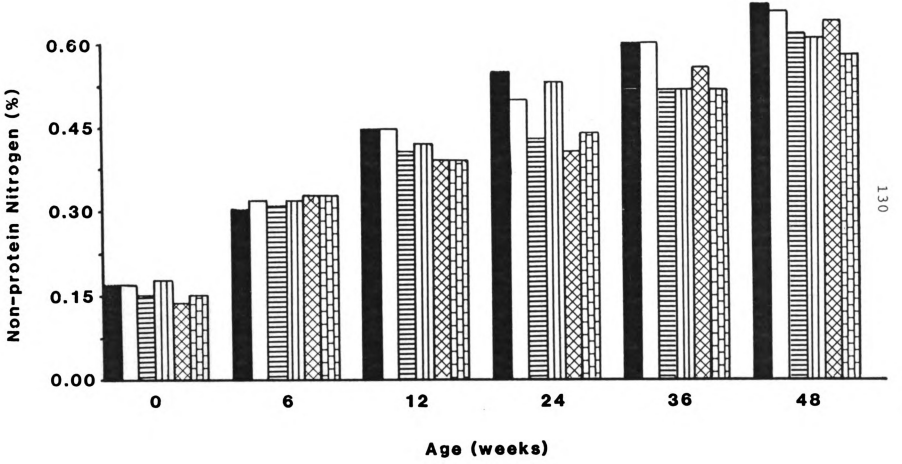
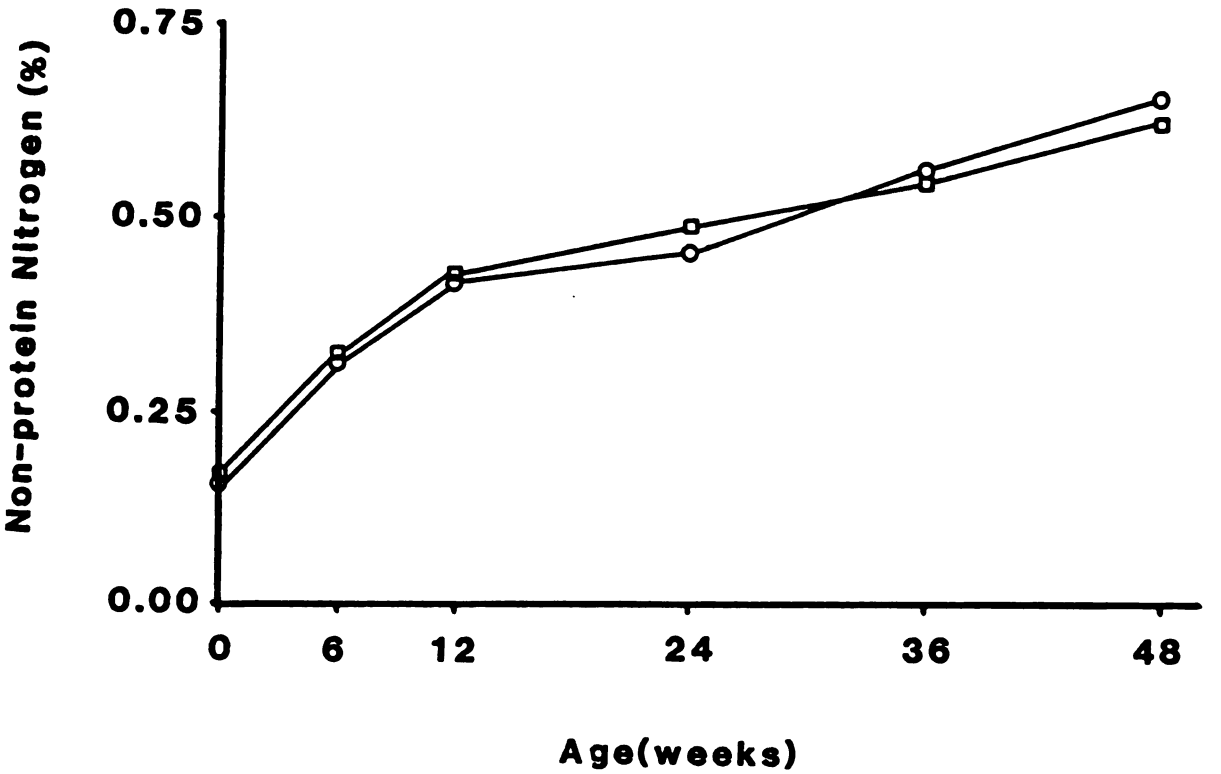

0.60
0.45
0.30
0.15
0.00
Non-protein Nitrogen (%)
0
6
12
24
36
48
Age (weeks)

Figure 14b. The effects of ripening on the non-protein nitrogen content of Cheddar cheese manufactured from control (o) and seeded (□) milk stored for 1,3 and 5 days.


0.75
0.50
0.25
0.00
Non-protein Nitrogen (%)
0
6
12
24
36
48
Age(weeks)

proteinases to degrade both α_s- and β-caseins (Law, 1978; Mills and Thomas, 1980; Visser and deGroot-Mostert, 1977). The extra enzyme content as a result of seeding may have contributed to the higher levels of NCN in the seeded milk cheese.

Peptidase activity and the resultant free amino acid increases have been ascribed to lactic cultures during the aging of cheeses (Visser, 1977b; O'Keefe et al., 1976). If all of the enzymes of the seeded lactic culture survived pasteurization one would have expected higher levels of NPN in the seeded milk cheese which was not the case. The peptidases may not have been as heat stable as the cell wall-bound extracellular proteinases, which would have resulted in relatively equal amounts of peptidase activity and subsequently equal levels of NPN in control and seeded milk cheese.

Electrophoretic Analysis

The proteolytic activity in the cheese during ripening was examined using discontinuous polyacrylamide gel electrophoresis. Densitometry was performed on the gels in an attempt to quantitate the relative changes in the casein composition of the cheese over the ripening interval. A standard whole casein sample was run with each batch of cheese samples to allow the identification of the major

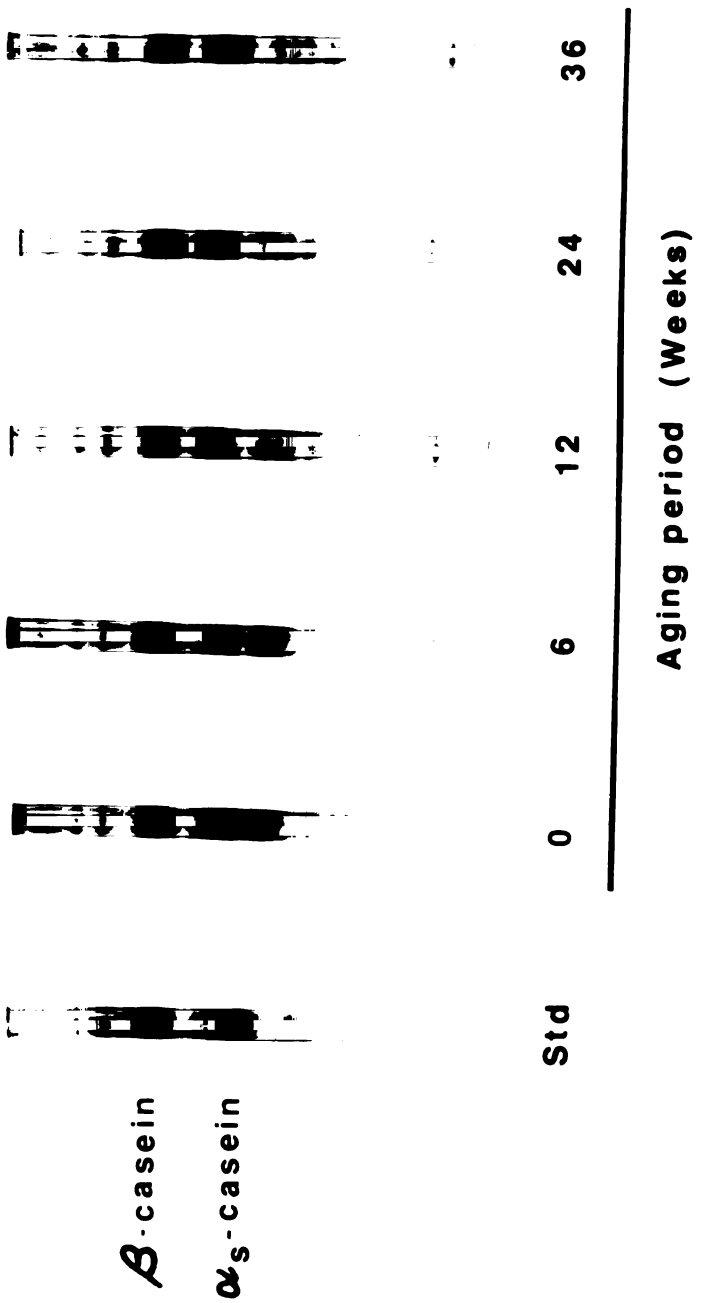
casein components, α_s- and β-casein. These were identified by comparisons of relative mobilities and densitogram shapes and areas.

The electrophoretic gels for the second trial were photographed with a casein standard and are displayed in figures 15, 17, 19, 21, 23, and 25. They are arranged so that each of the six possible days of storage and seeding treatment combinations showed 5 gels representing changes in casein patterns over 36 weeks of ripening. Figures 16, 18, 20, 22, 24, and 26 present densitograms for the displayed gels. No significant pattern differences were apparent between trials or between treatment combinations, therefore, these gels are considered representative of the experiment.

Differences in patterns as affected by aging were discernable. α_{s1}-Casein, as represented by peak 2 on the densitograms, showed rapid degradation and was an unresolved peak after 6 weeks. Because of the difficulty in α_{s1}-casein, an α_s-casein region was defined as indicated on the densitograms by the letter A with brackets defining the borders of the region. The densitograms indicate a decrease in the area of region A of 40-60% at six weeks (Figure 27). These sharp decreases were followed by small changes in the area of region A during the 12-36 weeks ripening.

Peak 1 was probably α_{s1}-I casein as described by Visser and deGroot-Mostert (1977) and showed an increased area after 6 weeks. The opposite was observed for α_{s1}-casein. At 12 weeks, the area constituting Peak 1 declined, accompanied

Figure 15. Disc-PAGE (9% T) patterns of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening and of whole casein standard (Std.). (Trial #2, Day 1 - Control)


β-casein
αs-casein
Std
0
6
12
24
36
Aging period (Weeks)

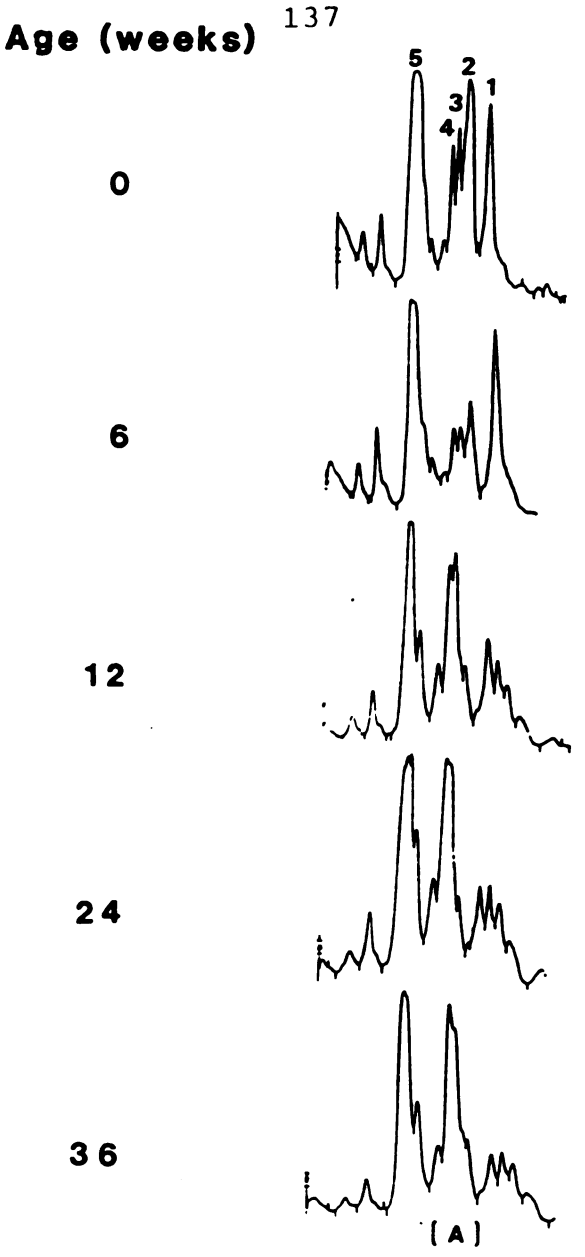
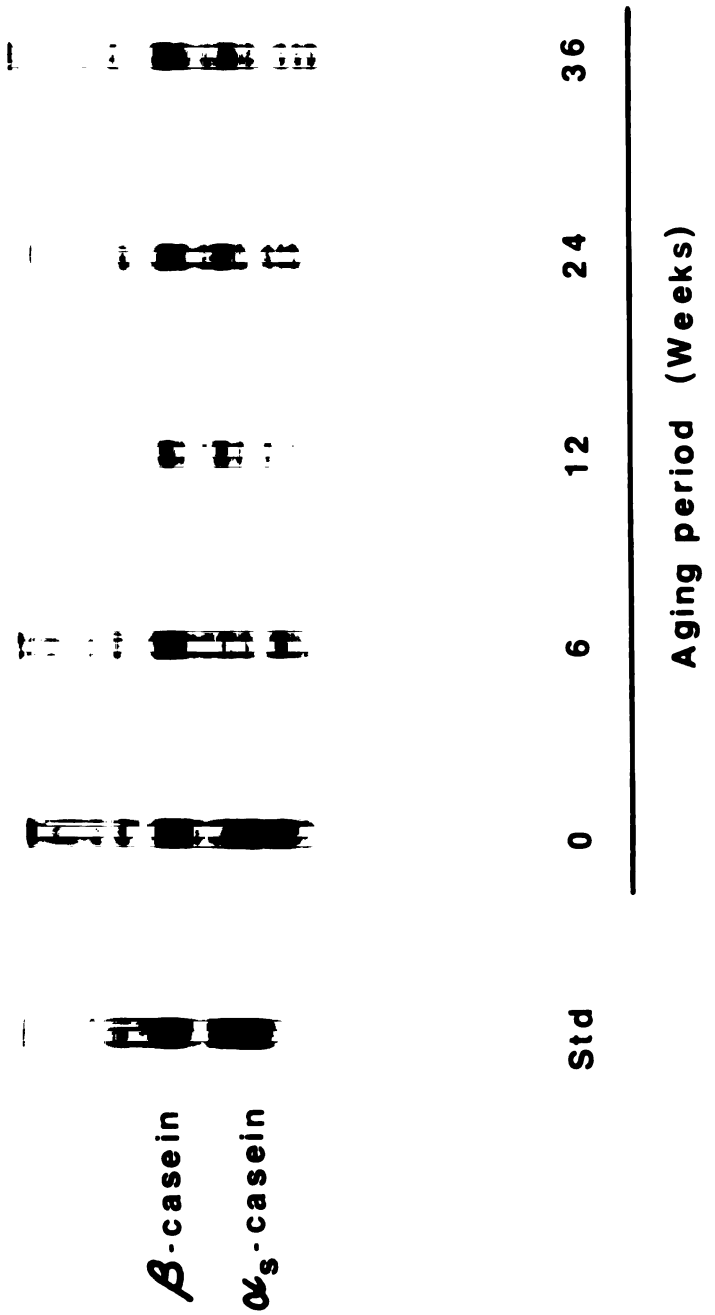


Figure 16. Densitometry patterns of electrophoretic gels of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening. (Trial #2, Day 1 - Control)

Figure 17. Disc-PAGE (9% T) patterns of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening and of whole casein standard (Std.). (Trial #2 - Day 1 - Seeded)


β-casein
α_s-casein
Std
0
6
12
24
36
Aging period (Weeks)

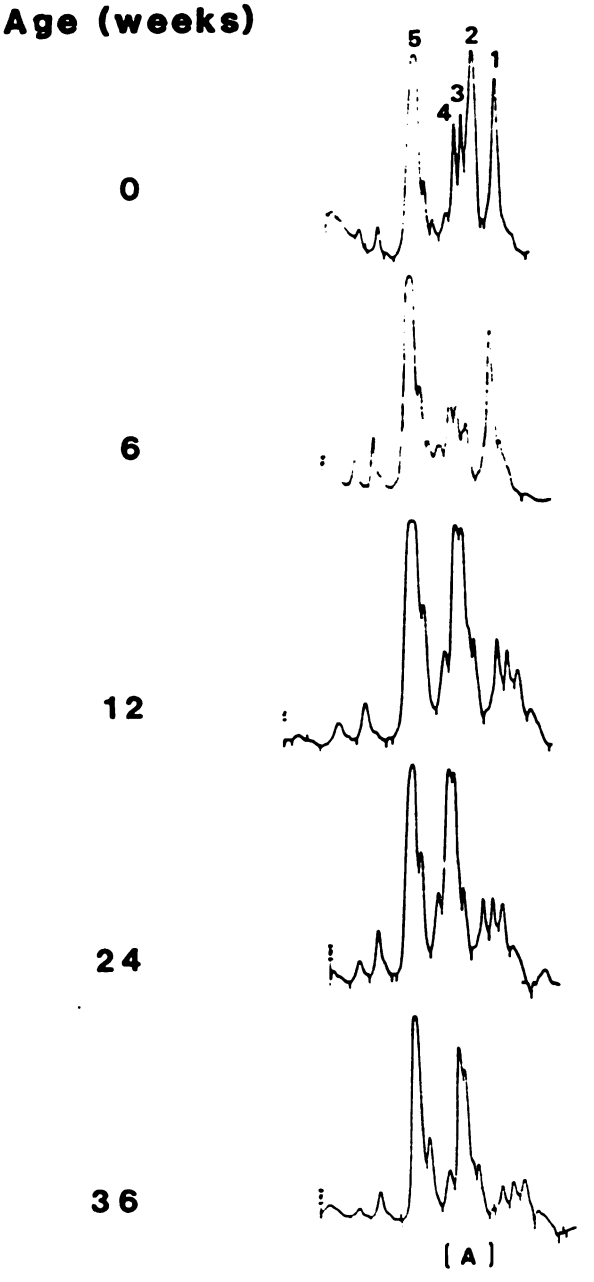
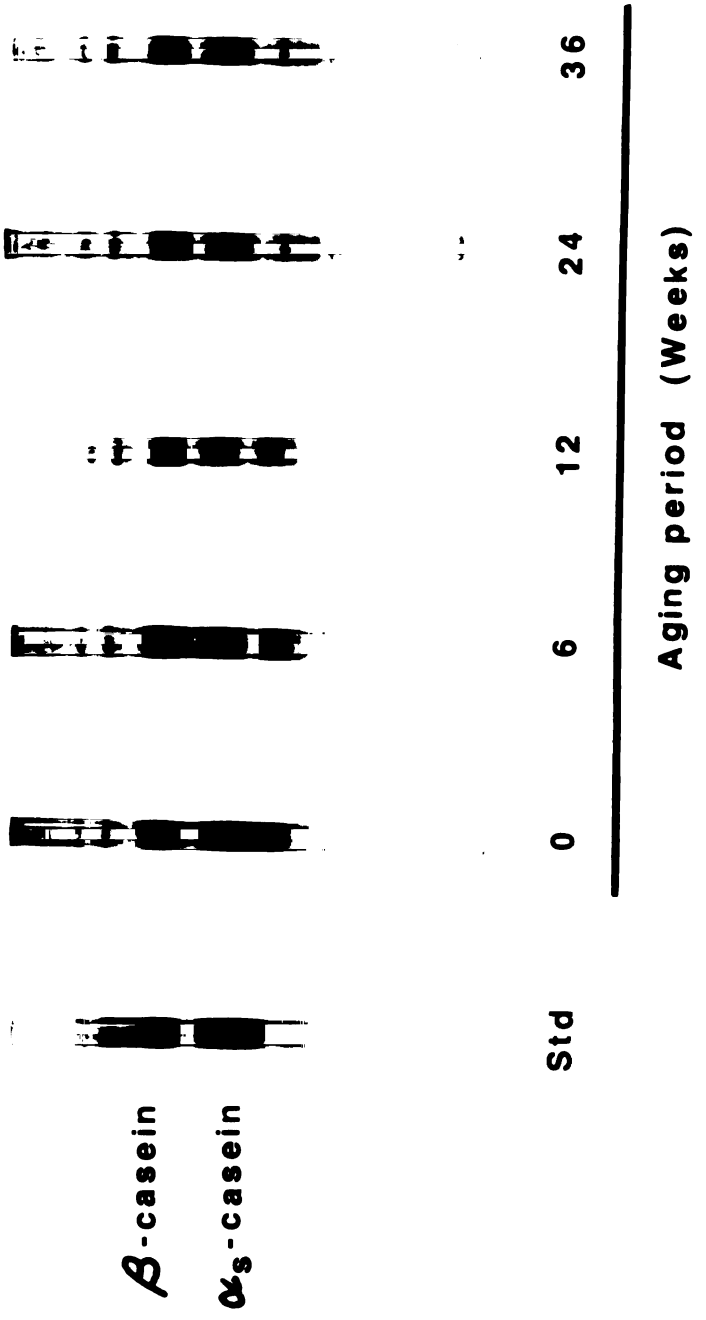


Figure 18. Densitometry patterns of electrophoretic gels of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening. (Trial #2, Day 1 - Seeded)

Figure 19. Disc-PAGE (9% T) patterns of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening and of whole casein standard (Std.). (Trial #2 - Day 3 - Control)


β-casein
αs-casein
Std
0
6
12
24
36
Aging period (Weeks)

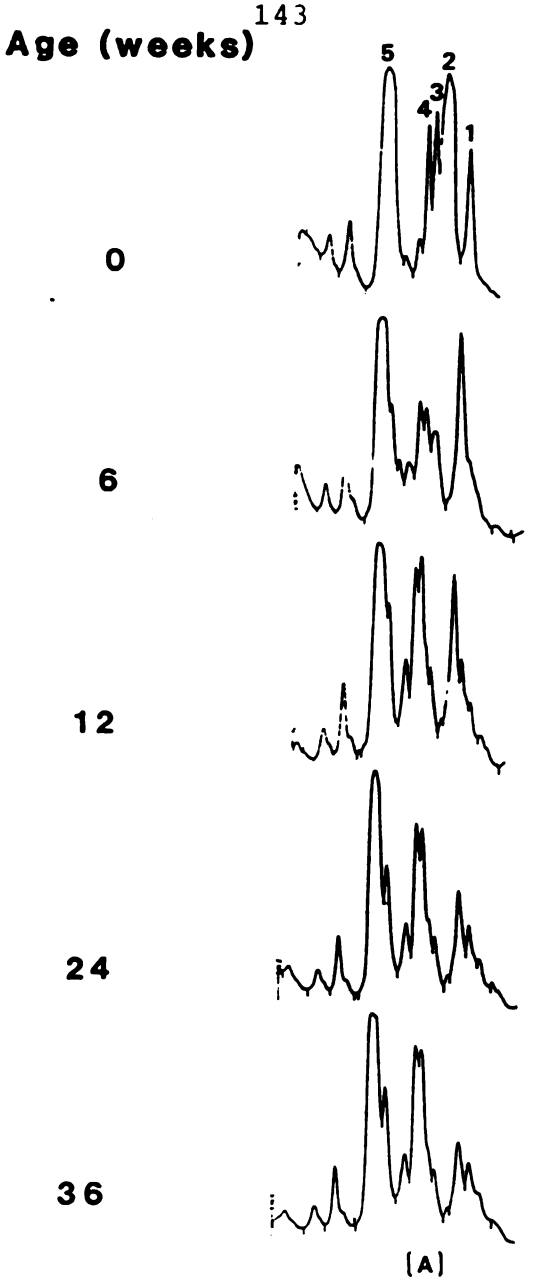
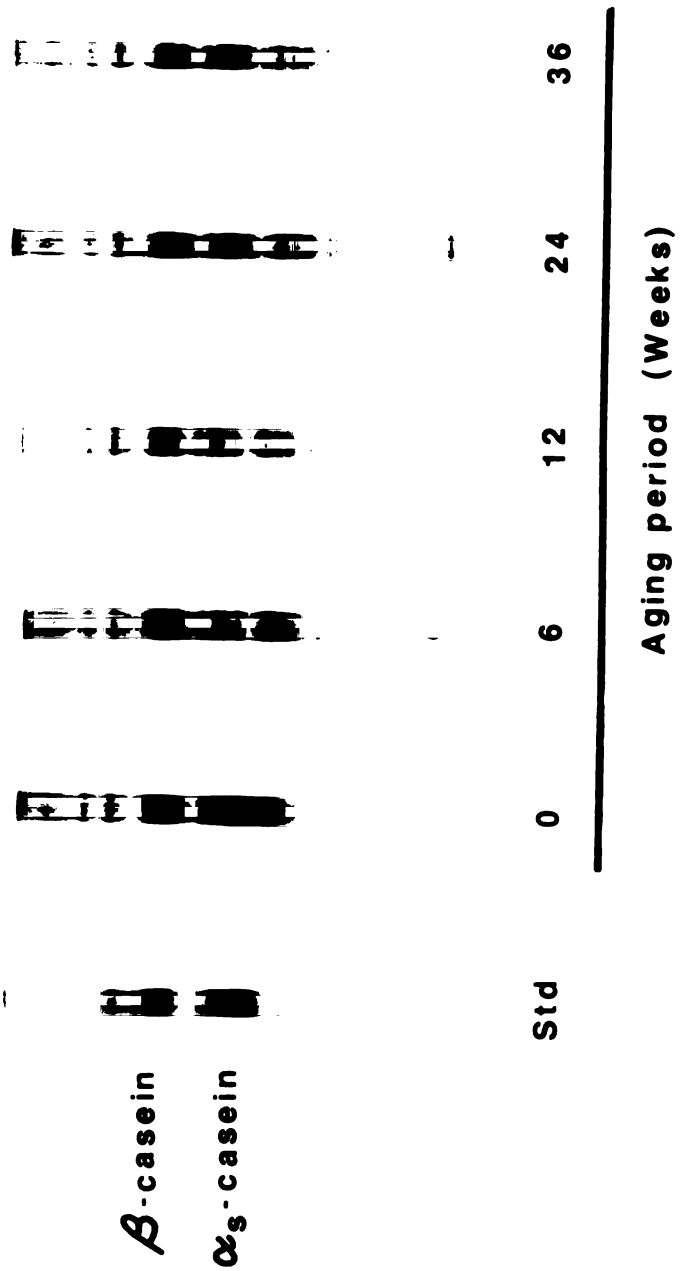


Figure 20. Densitometry patterns of electrophoretic gels of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening. (Trial #2, Day 3 - Control)

Figure 21. Disc-PAGE (9% T) patterns of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening and of whole casein standard (Std.). (Trial #2 - Day 3 - Seeded)


β-casein
α_s-casein
Std
0
6
12
24
36
Aging period (Weeks)

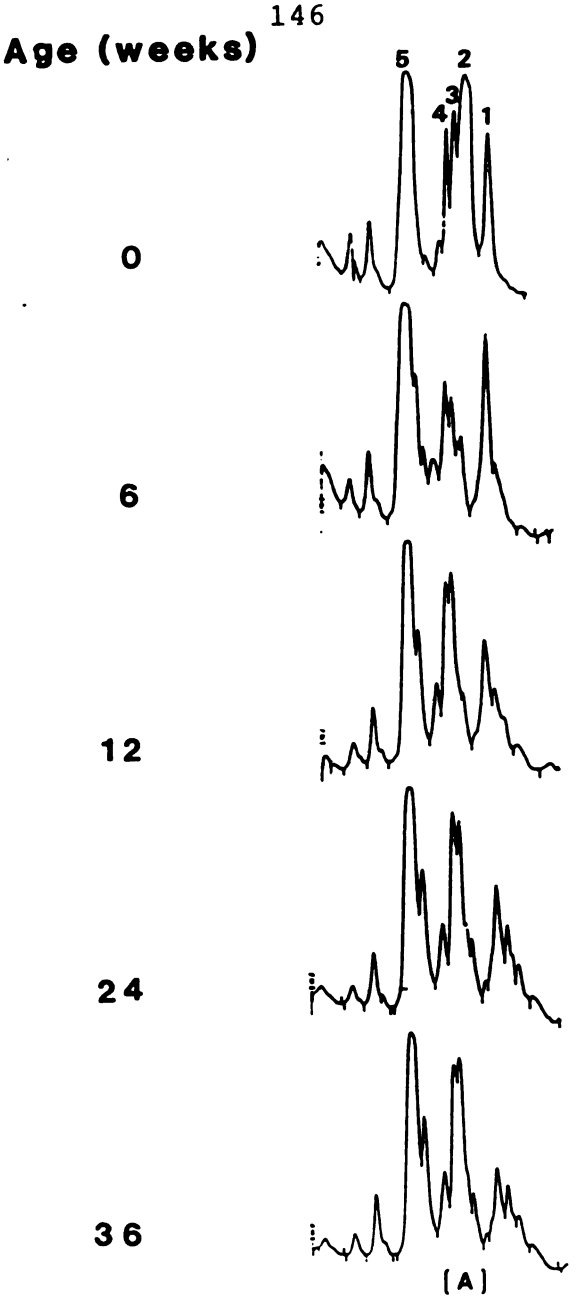
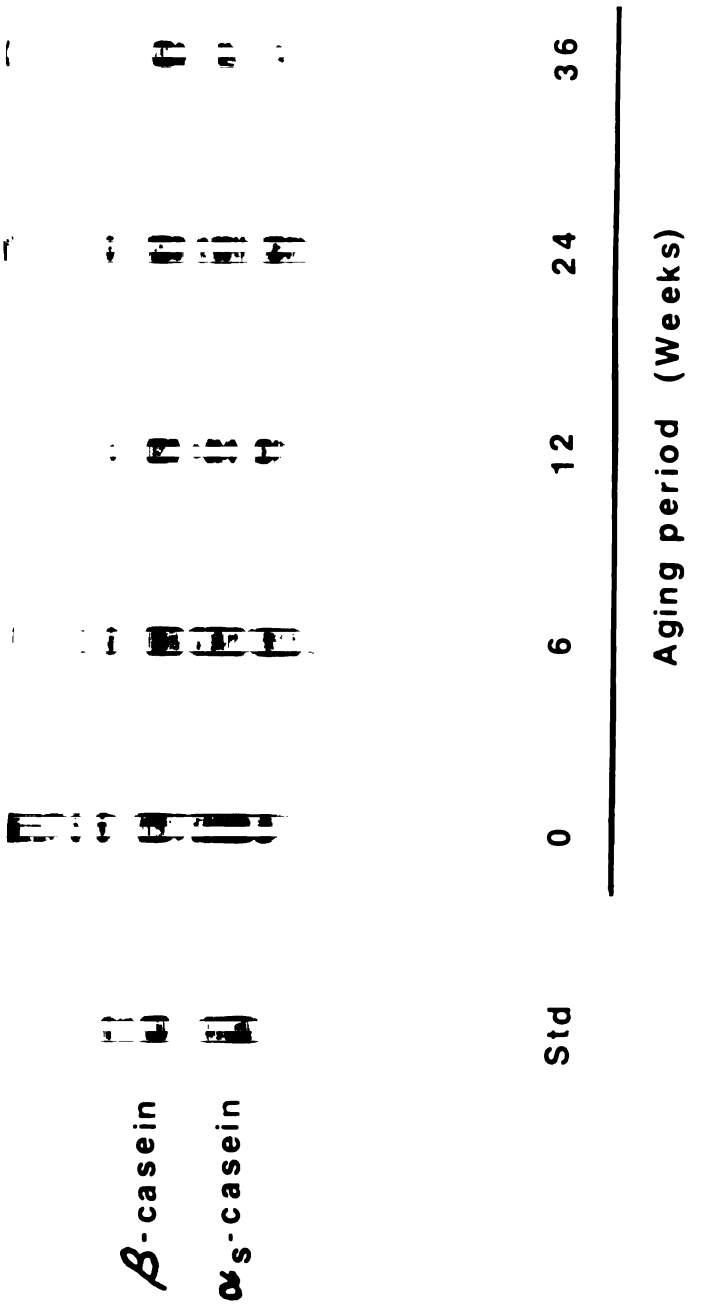


Figure 22. Densitometry patterns of electrophoretic gels of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening. (Trial #2, Day 3 - Seeded)

Figure 23. Disc-PAGE (9% T) patterns of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening and of whole casein standard (Std.). (Trial #2 - Day 5 - Control)


β-casein
αs-casein
Std
0
6
12
24
36
Aging period (Weeks)

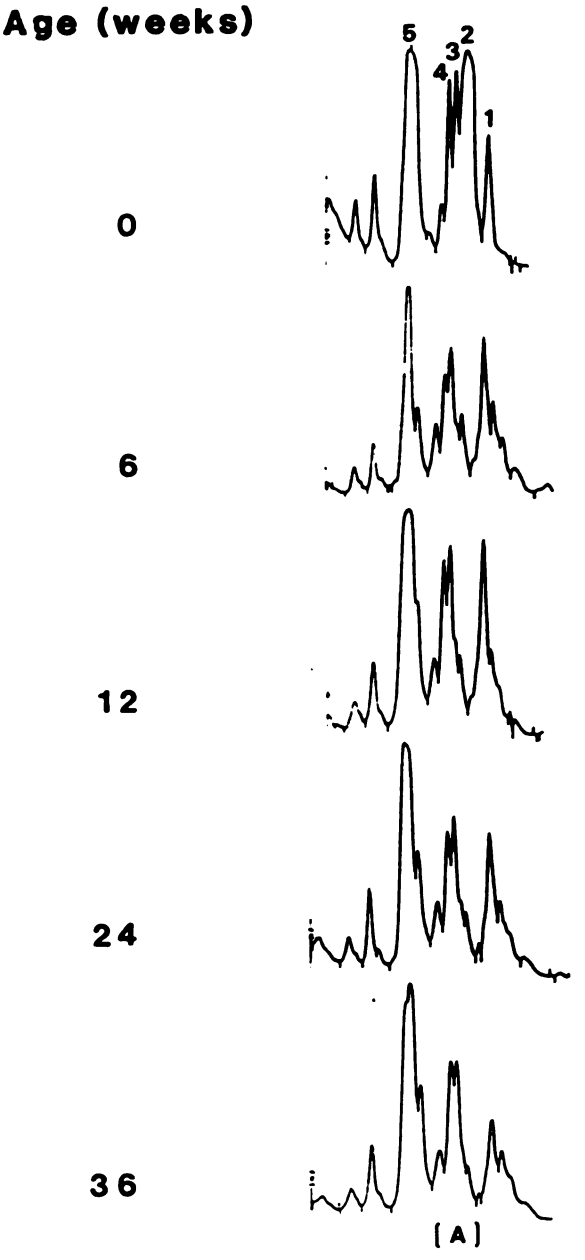
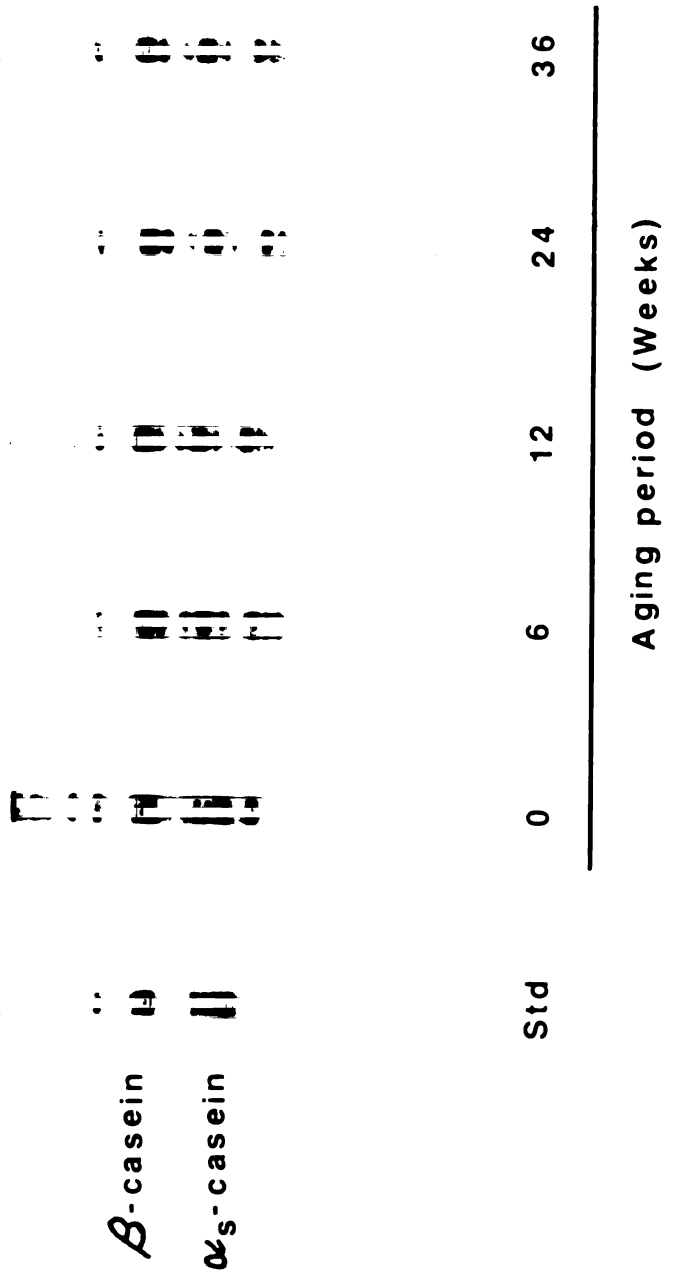


Figure 24. Densitometry patterns of electrophoretic gels of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening. (Trial #2, Day 5 - Control)

Figure 25. Disc-PAGE (9% T) patterns of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening and of whole casein standard (Std.). (Trial #2 - Day 5 - Seeded)


β-casein
αs-casein
Std
0
6
12
24
36
Aging period (Weeks)

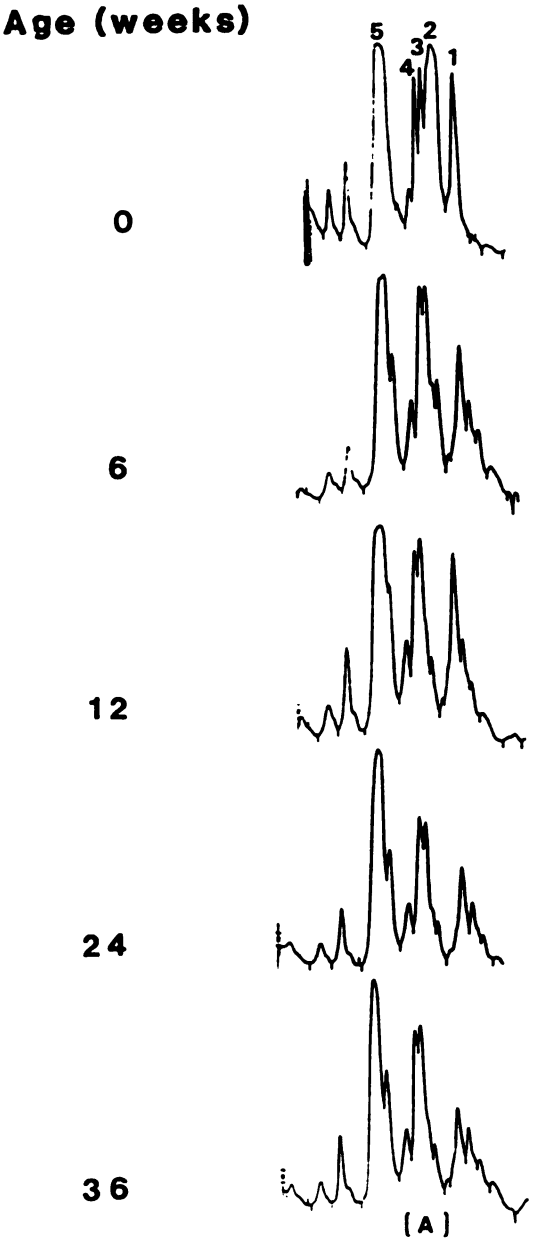
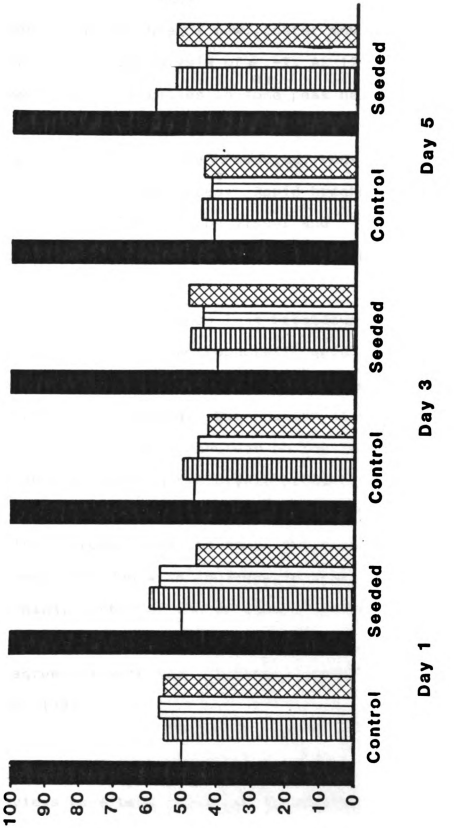


Figure 26. Densitometry patterns of electrophoretic gels of Cheddar cheese at 0,6,12,24 and 36 weeks of ripening. (Trial #2, Day 5 - Seeded)

Figure 27. Mean densitometric area of α_{s}-casein region (peaks 2-4) of Disc-PAGE patterns of Cheddar cheese at 0 (■), 6 (□), 12 (▤), 24 (▥) and 36 (▩) weeks.


Retention (%)
100
90
80
70
60
50
40
30
20
10
0
Control
Seeded
Control
Seeded
Control
Seeded
Day 1
Day 3
Day 5

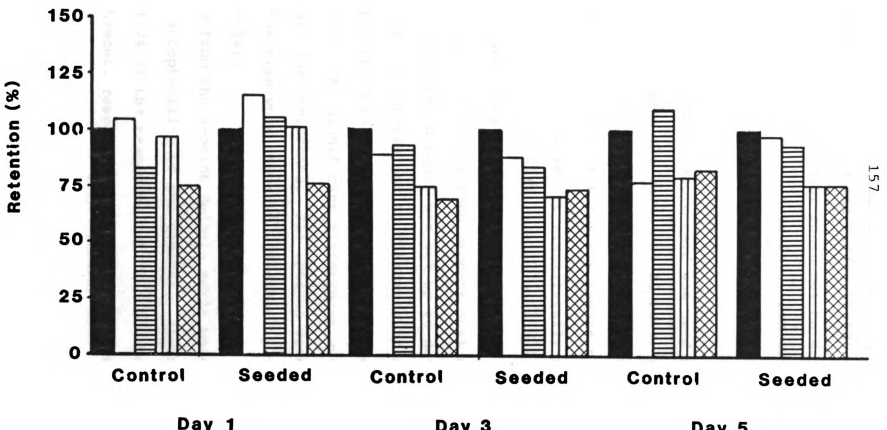
accompanied by the appearance of new, faster moving zones, indicating the further hydrolysis of α_{s1}-I. At 12 weeks, region A showed slight increases in some peak heights, indicating products of hydrolysis which had relative mobilities equal or close to the α_s-caseins, thus confounding identification of individual zones. Rapid breakdown of α_{s1}-casein has been demonstrated by Visser and deGroot-Mostert (1977), Ledford et al. (1966) and Noomen (1978a).

β-casein (Peak 5) showed much less breakdown over the 36 week period as recorded by electrophoretic gels. Increases in peak area of -casein appeared in some of the gels during the first 12 weeks of ripening. Examination of the densitograms revealed a small shoulder on the leading edge of peak 5 which indicated an hydrolysis product with a relative mobility slightly faster than β-casein. The amount of the hydrolysis product was too small for resolution therefore it was included with the β-casein peak. After 36 weeks of ripening, 75-80% of the original β-casein was retained showing less hydrolysis than α_s-caseins (Figure 28). This was in agreement with the literature (Ledford et al., 1966; DeWille, 1983).

Organoleptic Evaluation

Many reviews have been published describing the identification of the chemical components of Cheddar cheese flavor (Mabbitt, 1961; Marth, 1963; Forss and Patton, 1966;

Figure 28. Mean densitometric area of β-casein region (peak 5) of Disc-PAGE patterns of Cheddar cheese at 0 (■), 6 (□), 12 (▤), 24 (▥) and 36 (▩) weeks.


150
125
100
75
50
25
0
Retention (%)
Control
Seeded
Control
Seeded
Control
Seeded
Day 1
Day 3
Day 5

Reiter and Sharpe, 1971; Aston and Dulley, 1982). The common conclusion was that cheese flavor could not be determined completely by chemical methods, therefore, sensory analysis of the product was used in the present study as the final comparative tool.

The ADSA Cheddar cheese score card was used by three expert judges to evaluate the cheese at 12 weeks of ripening. The mean flavor of the 4 trials is recorded in Table 23 and showed lower scores for the seeded milk cheeses. The differences were not statistically significant and both control and seeded milk cheeses were debited most commonly for slight bitterness.

The final approval of any food product must come from the consumer, therefore, a panel was set up to determine the acceptability of the cheeses and to determine if any of the cheeses were preferred based on day of manufacture and seeding treatment. No significant differences were found in the ranking test for preference. All cheeses were preferred about 50% of the time when compared individually to other samples (Table 24).

Responses from the 5-point Hedonic scale were used to determine the acceptability of the cheeses. Seventy-one percent and 71.7% of the responses recorded for control and seeded milk cheeses, respectively, were on the Like or Slightly Like points of the scale (Table 25). This indicated an acceptable product from the consumer viewpoint.

Table 23. ADSA Flavor Scores - 12 Weeks.

	Days of Milk Storage		
Sample	1	3	5
Control ave.	7.75	8.00	8.00
st. dev.	1.06	0.74	0.60
Seeded ave.	7.33	7.17	7.00
st. dev.	1.15	0.83	0.85

Table 24. Results of ranking by consumer panel.

Cheese	Preferred above all other samples
	%
Day 1 Control	44.8
Day 1 Seeded	53.1
Day 3 Control	55.1
Day 3 Seeded	44.5
Day 5 Control	51.4
Day 5 Seeded	51.1

Table 25. Results of acceptability evaluation by consumer panel.

	5-Point Hedonic Scale				
Sample	Like	Slightly Like	Neutral	Slightly Dislike	Dislike
Control	303	209	120	64	25
Treated	316	194	96	74	31

SUMMARY AND CONCLUSIONS

The inhibitory activity of the lactic culture, used to seed milk at 5×10^6 cfu/ml, could not be evaluated due to the low numbers of psychrotrophic bacteria present in three of the four trials. Therefore, the conclusions of this study will be limited to the effects of the seeding and storage of milk on compositional and organoleptic quality.

The seeded milk showed an increase in acidity as measured by pH and titratable acidity. Increased firmness of rennet curd was correlated with the higher acidities and cheeses with higher moisture contents were manufactured from the seeded milk. No other differences were detectable in the control and seeded milk or their corresponding whey.

The yields of control and seeded cheeses showed a non-significant trend toward higher levels on the third and fifth days of manufacture. The reassociation of soluble phase -casein with the micelle during extended storage was the probable cause for the increased yields. Statistical analysis of the yields and solids recoveries were hampered by the small size of the batches. Any further work should be done with a batch size of at least 2000 kg to reduce the error due to unavoidable product losses.

Both the control and seeded cheeses showed typical rapid decreases in Lactic Bacteria Counts during ripening. An increase in pH between 24 and 36 weeks may be partially due to a corresponding decrease in FFA. The lower levels of FFA may have resulted from a shift in equilibrium towards the formation of esters. The ripening period of 36-48 weeks showed a reversal of the pH and FFA trends and may have been caused by the release of lipolytic enzymes from lysing lactobacilli.

Non-casein nitrogen increased at a decreasing rate during ripening and levels in the seeded cheeses were significantly higher. Cell wall-bound extracellular proteinases of the lactic culture may have survived the pasteurization thereby increasing the production of NCN. Non-protein nitrogen also increased at a decreasing rate during ripening but showed no treatment differences. The intracellular peptidases responsible for the production of NPN fractions may have been more sensitive to the heat treatment causing the control and seeded cheeses to have approximately equivalent amounts of peptidase activity.

No significant differences were found in the electrophoretic (Disc-PAGE) patterns of the cheeses based on treatment. During the ripening period, α_s-caseins were hydrolyzed to a much greater extent than β-casein. The area of the β-casein peak remained at 75-80% of its original area after 36 weeks of ripening. The α_s-casein peak area reduced to an insignigicant level within the first 12 weeks. This

was accompanied by the appearance of a large, faster α_{s1} I-zone, which subsequently hydrolyzed into smaller, faster zones during the following weeks. Two peaks in the α_s-casein region that were slower than α_{s1}-casein showed increased areas during ripening as a result of the accumultion of hydrolysis products.

The ultimate test for a cheese is the acceptance by the consumer. The results of the consumer panel indicated a high degree of acceptability for both control and seeded cheeses.

Very few differences were found in the cheeses based on seeding or days of storage, therefore, one could state that the seeding of raw milk with lactic cultures during storage has no detrimental effects on the yield or quality of Cheddar cheese. More work is needed to evaluate the possibility of accelerated ripening due to extra enzyme activity and to identify strains of lactic streptococci that are effective inhibitors to psychrotrophic bacteria.

APPENDIX

APPENDIX

Electrophoresis Solutions

The stock solutions needed were:

1. Running gel buffer, pH 8.9, 0.380 M Tris HCl. It was prepared by dissolving a 4.6018 g of Tris (hydroxymethyl) aminomethane in about 95.0 ml distilled water; 42.0 g of urea were added to make the buffer 7 M. The pH was adjusted to 8.9 with concentrated HCl and the volume was made to 100.0 ml with distilled water.
2. Stacking gel buffer, pH 6.7, 0.062 M Tris-HCl, 7 M urea, was prepared y dissolving 0.7508 g of Tris in about 95.0 ml distilled water; 42.0 g of urea were added to make the buffer 7 M. The pH was adjusted to 6.7 with concentrated HCl and volume was made to 100.0 ml with distilled water.
3. Electrode buffer, pH 8.3, 0.046 M Tris glycine, was prepared by dissolving 16.71 g of Tris in about 2100 ml distilled water. The pH was adjusted to 8.3 with 2 M glycine solution and the volume was made to 3000 ml with distilled water.
4. Running gel solution, 25% (w/v) acrylamide solution, was prepared by dissolving 24.83 g of acrylamide monomer and 0.64 g of NN-Methylenebisacrylamide (BIS)

in 75 ml of the running gel buffer and making it to 100 ml with the same buffer. This provided a stock solution with 25% total acrylamide.

5. Stacking gel solution, 6.25% (w/v) acrylamide solution, was prepared by dissolving 5 g of acrylamide monomer and 1.25 g of BIS in 75 ml of the stacking gel buffer and making it to 100 ml with the same buffer.
6. Ammonium persulfate solution, 5% (w/v) 7 M urea, was prepared by adding 0.625 g ammonium persulfate and 5.25 g urea in 12.5 ml of distilled water. The solution was prepared every two days.
7. N,N,N',N'-tetramethylethylene diamine (TEMED).
8. Bromophenol blue, 1% (w/v) solution made in stacking gel buffer.
9. Saturated sucrose solution made in stacking gel buffer.
10. Stainer solution. It contained 25% (v/v) Isopropanol, 10% (w/v) acetic acid and 0.05% (w/v) coumassie brilliant blue R-250 in distilled water.
11. Destained solution. It contained 5% (v/v) acetic acid and 10% (v/v) isopropanol in distilled water.

All solutions were filtered before using.

Gel Preparation

1. The dry tubes were marked with a felt-tip pen at distances 10.0 and 11.6 cm from the bottom.

2. The bottom of each tube was fitted with a small square of parafilm. Tubes were then placed in a leveled rack.
3. A gel solution of the desired concentration (9%) was prepared by combining 9 ml of running gel solution and 15.7 ml running gel buffer to give a final volume of 24.7 ml.
4. To this gel solution, 20 l of TEMED and 0.3 ml of ammonium persulfate solution were added.
5. The gel solution was transferred to the glass tubes with a syringe fitted with an 18 gauge needle. Each tube was filled to the 10.0 cm mark, carefully overlayed with distilled water, and allowed to polymerize overnight.
6. After polymerization of the running gel, the water layer was removed and the top of the running gel was rinsed with stacking gel buffer. The buffer was removed from the running gel.
7. The stacking gel was prepared by mixing 5.0 ml of the stacking gel solution with 1 g of sucrose. The volume of the solution was made to 10.0 ml with stacking gel buffer; 40 l of ammonium persulfate and 10 l of TEMED were added.
8. Each tube was filled to the 11.6 cm mark with stacking gel, overlayed with water, and allowed to polymerize for one hr.

Electrophoretic Conditions and Staining Procedure

1. The glass tubes containing the gels were transferred to the electrophoretic apparatus and the water was removed from the top of stacking gels. The anodic and cathodic buffer reservoirs were filled with the electrode buffer. The protein samples of 20 l were then carefully layered on top of the stacking gels.
2. Electrophoresis was conducted at 1 mA/tube for 10 min. After that a constant current of 2 mA/tube for the cheese and casein samples was maintained. Electrophoresis was concluded when the marker dye had migrated to the bottom of the gels, about 3 hr.
3. The gels were removed from the glass tubes and stained in 0.05% (w/v) coumassie brilliant blue R-250 overnight.
4. Destaining was performed for 2 days by the diffusion method in the destainer solution.

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