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IN LATIN AMERICAN WHITE CHEESE

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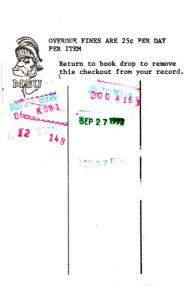
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# FLAVOR AND TEXTURE DEVELOPMENT IN LATIN AMERICAN WHITE CHEESE

Ву

Normanella Torres

## A THESIS

Submitted to

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#### **ABSTRACT**

# FLAVOR AND TEXTURE DEVELOPMENT IN LATIN AMERICAN WHITE CHEESE

By

#### Normanella Torres

Eighteen 195-kg lots of whole milk were heated to 82 C and precipitated by addition of citric acid. To modify flavor and texture, lactic culture, yogurt culture and lipase enzymes were applied to the curd before pressing. Vacuum packed blocks were ripened at 10 C. Physico-chemical changes were measured over a 12 wk period. In all cases, cheese composition was similar. Increase in firmness (Kramer-Shear) was noticed in all treated and untreated cheeses as a function of time. Maximum protein breakdown was observed with yogurt culture after 8 wk of ripening. pH values decreased from 5.21 to 4.86. Free fatty acids and volatile acids steadily increased, especially in lipase treated samples.

Sensory evaluations showed statistically significant differences between treated cheese and control. Four-week ripened cheese containing yogurt cultures was preferred. Latin American White cheese was observed to improve its organoleptic characteristics by addition of yogurt culture, lipase enzymes and lactic culture.

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#### INTRODUCTION

Total milk (cow, buffalo, sheep and goat) used by humans for direct consumption or prepared products (cheese, ghee, butter, yogurt, ice cream, and other manufactured products) is estimated at 450 to 455 million metric tons (MT) per year (FAO, 1977). Milk and milk products provide about 18,550 million MT or 35% of all animal protein and 364,640 billion megacalories or 35% of all energy from animal sources (McDowell, 1977). The average daily per capita protein consumption for animal products of all types is 55.6 g in developed countries and only 11.2 g in the developing countries (FAO, 1977). Per capita daily consumption of milk and milk products in developed areas of the world varies from .5 to .8 liter but from almost nil to .3 liter in the developing areas (McDowell, 1977).

The preservation of milk and milk products is critical in warm countries due to the rapid deterioration of milk at high temperatures together with the need for good means of providing nutrition. The food value of milk has been preserved in the form of cheese in many parts of the world. White unripened cheese in which the protein is precipitated using rennet or acid is known as Kareish in Egypt, Chhana in India, Armavir in the Western Caucasus,

Zsirpi in the Himalayas, Feta in the Balkans and Queso Criollo, Queso del Pais, Queso Llanero in Latin America (Sanders, 1953). In spite of wide differences in the manufacturing process in each country, all these cheeses belong to the same group. Typically, the cheese is made without starter cultures. Cheese curd is obtained by direct acidification of whole milk at 82 C with a food-grade acid, such as lactic, tartaric, citric, phosphoric, or acetic acids (Siapantas and Kosikowski, 1973a). In some regions these acids may be replaced with yogurt, vinegar, lemon juice or other fruit juices.

Queso blanco type cheeses of Latin America possess several nutritional advantages. They are relatively high in mineral and protein, and low in lactose content. This is especially important in areas facing the lactose intolerance problem. Because of low moisture and high salt content, the white cheese has good shelf-life and lends itself to distribution for marketing purposes. Furthermore, this product has always formed part of the dietary pattern of these countries. Therefore, there is no social or cultural problem involved with its use. Other advantages include relatively simple equipment and skill required in making the cheese.

In the United States per capita sales of cheese have increased 57% during 1967-1977 (Milk Industry Foundation, 1978). New cheese varieties and products should facilitate further growth in this area. Latin American white

cheese appears to offer a considerable potential as a product or a food ingredient (Chandan and Marin, 1978). In addition, the white cheese offers economic advantages due to its relatively short ripening period, high yield and exceptional functionality, i.e. frying ability without melting (Chandan et al., 1979).

The present work was undertaken to develop a new flavor and texture in Latin American white cheese by direct treatment of the cooled curd with yogurt culture, lactic culture or lipase enzymes. Another objective of this study was to acquire information on flavor development in this cheese by investigating the following changes occurring during a ripening period of 12 wk:

- 1. Hydrogen ion concentration or pH.
- 2. Body firmness by Kramer shear measurements.
- Production of free fatty acids as a measure of lipid breakdown.
- 4. Protein degradation by soluble nitrogen assay.
- 5. Monitoring volatile acids as indicators of general cheese flavor development.

#### LITERATURE REVIEW

Cheese is a highly nutritious and palatable food. It consists of the casein and most of the fat, insoluble salts and colloidal material, together with part of the lactose, whey proteins, soluble salts, vitamins and other minor components of milk (Wong, 1974). The proportion of milk constituents that go in to cheese and whey as listed by Chandan (1979b) is shown in Table 1. Of the other important nutrients, two thirds of the calcium, most of the vitamin A, one fourth of the riboflavin and about one sixth of the thiamin remain in the curd. Except for fresh-curd cheese, the small amount of vitamin C present in the original curd is rapidly lost in the initial stages of ripening. The other vitamins are, on the whole, stable during ripening and storage. Moreover, bacterial and mold

Table 1. Partition of major milk constituents into Cheddar cheese and whey.\*

Constitutent	<pre>% Retained in Cheese</pre>	% Retained in Whey
Water	4	96
Milk Fat	93	7
Milk Protein	75	25
- Casein	96	4
- Whey	7	93
Minerals	35	65
Lactose	3	97

Chandan, R. C. (1979).

activity leads to synthesis of several vitamins of the B complex group (Kon, 1972).

## Classification of Cheeses

Throughout the world, more than 400 cheeses with more than 800 names have been described (Sanders, 1953). However, the manufacturing processes for many are quite similar or identical. Probably, there are only 18 distinct types of natural cheese. Cheeses have been classified according to texture, moisture, age and ripening agent. Most cheeses fall within the classification stated by Day (1967):

### I. Natural

- (A) Unripened
  - (1) Low fat, e.g., Cottage cheese, Baker's cheese
  - (2) High fat, e.g., Cream, Neufchatel cheese
- (B) Ripened
  - (1) Hard grating cheese, e.g., Romano, Parmesan, Asiago Old
  - (2) Hard, e.g., Cheddar, Swiss, Gruyere,
    Provolone, Gouda
  - (3) Semisoft, e.g., Roquefort, Blue, Gorgonzola, Brick, Limburger
- (4) Soft, e.g., Camembert, Brie, Liederkranz
  II. Whey cheeses, e.g., Ricotta, Mysost

## Latin American White Cheese

Latin American White cheese is found throughout South and Central America, Mexico and the Caribbean islands. Ιt is made from whole, partly skimmed, or skimmilk or from whole milk with cream or skimmilk added. Much of this cheese is eaten fresh, within a day or two after it is made, either without being pressed or after pressing. Some of the pressed cheese is held for periods ranging from two weeks to two months or more (Wilster, 1974). This cheese is known by many different local names (Sanders, 1953): fresh skimmilk cottage-type cheese is called Queso de Puna in Puerto Rico; Queso Fresco (fresh cheese) in El Salvador and Venezuela. Other skimmilk cheeses are called, Queso Llanero, Queso de Maracay and Queso de Perija in Venezuela and Oueso Descremado or Oueso Huloso in Costa Rica. Cheese made from whole or partly skimmed milk in Mexico is called Panela and Oueso de Prensa (pressed cheese) in El Salvador and Venezuela. In Puerto Rico, these cheeses are called Queso del Pais or Queso de La Tierra and in Colombia, Queso de Estera. Cheese made from whole milk, heavily salted, pressed, cured for 1 to 2 months, and used as a grating cheese is called Queso de Bagaces in Costa Rica. Cheese made from whole milk, salted and pressed lightly, and cured for 2 weeks to 2 months is called Queso de Crema (cream cheese) in Panama.

Latin American White cheese defined in this study is a white creamy cheese, highly salted and acid in flavor

(Kosikowski, 1977). Its texture and body resembles young high-moisture Cheddar, and it has good slicing properties. Chandan et al. (1979) reported on its ability to resist melting at frying temperatures. The pressed cheese is hard, crumbly, with a salty flavor and rather open texture (Wilster, 1974).

# Methods of Manufacturing Latin American White Cheese

Queso Blanco type cheese in Latin American countries is made on the farms as well as in the industrial plants. The farm product is a soft or semihard cheese and is usually called "Queso de Matera". The industry product is always soft and is called "Queso Pasteurizado" (Escoda, 1973). Cheese made on the farm differs from that made by the industry in that it is made from raw milk and it has very high salt content (8-25%). Thus, it is drier than that made by the industry. Due to the distance between the farms and the centers of higher demand, the time elapsed between manufacture of the cheese and consumption is longer. Apparently some kind of ripening takes places in this product.

The general procedure for manufacture as stated by Davis (1976) is as follows: fresh, warm, raw milk is put into a wooden vat which often is a dugout "canoe" known as "canoa". Milk is curdled with commercial rennet in about 45 min. The coagulum is cut with a long knife crossways, or is broken by hand, and stirred for 20 to 30 min. After

letting the curd settle for 20-40 min, 5% of salt (in relation to milk) is added and the curd is left in this brine whey until the next morning. The whey is then drawn off and the curd is pressed in the bottom of the vat and ground afterwards. More salt is added to bring the salt content of the curd up to 6 or 7%. After the second salting, the milled curd is packed tightly into moulds and pressed.

More complete descriptions of some of the Latin American cheeses are given by Sanders (1953).

Queso de Cincho. It is also called Queso de Palma Metida. It is a sour-milk cheese made in Venezuela. It is spherical, 8 to 16 inches in diameter, and is wrapped in palm leaves.

Queso de Hoja. It is made in Puerto Rico. Fresh cow's milk is coagulated, the curd is cut into blocks about 6 inches square and 2 inches thick, and part of the whey is "drained" off. Then the blocks of curd are immersed in water or whey at 150 F. This treatment forms a tough layer of curd on the outside of the block. The curds are pressed with broad wooden paddle. Salt is sprinkled on the surface, and each piece of curd is folded in layers, wrapped in cloth, and squeezed to force out the whey. When cut, the thin layers of curd are distinct and look like leaves resting on each other.

Queso del Pais. It is a white pressed, semisoft, and perishable cheese made in Puerto Rico. Neither starter

nor rennet is used. The curd is coagulated with heat and acid, and is further neutralized with sodium bicarbonate.

Other white cheeses common in Venezuela and described by Eekhog-Stort (1976) are:

Queso de Mano. This cheese takes its name from the fact that the curd is worked by hand until the right consistency is achieved. Then it is put in layers in wooden molds where it remains for 24 hr. Young Queso de Mano is packed in banana leaves to retain moisture and give it a banana leaf aroma.

Queso Llanero. Fresh milk, with starter and salt is left in the cheese vat for 24 hr until it has coagulated. Curd is placed in molds and becomes hard and crumbly. It has a strong taste.

### Curd Formation

As may be seen from the examples above, the method of making Latin American White cheese varies in different countries. In the same country, different farms use different methods. Curd is obtained by the action of rennet or starter or by combining rennet and starter or by the addition of a food-grade acid including fruit juices or yogurt. Siapantas and Kosikowski (1973a) studied the manufacture of good quality Latin American White cheese using lactic, tartaric, citric, and phosphoric acid as coagulating agents. They reported that a high milk acidity leads to increased protein and fat losses in

whey. This loss was attributed to the formation of a very fine coagulum during heating.

Chandan et al. (1979) reported the production of Queso Blanco type cheese by direct acidification of whole milk to pH 4.7-4.8 with a food grade acid at 82 C and without use of rennet or a lactic culture. Other coagulants found to work are acid whey concentrates (Hirschl and Kosikow-ski, 1975), acetic acid (Siapantes and Kosikowski, 1965), lemon juice, vinegar and yogurt (Kosikowski, 1977).

According to Kosikowski (1977) most cheeses of the world are formed from one of three distinct curd precipitation patterns. In cheese varieties such as Queso Blanco types, Cottage or Cream, precipitation occurs at 82 C immediately or in 5 to 16 hr at room temperature as a result of an acid exerting a coagulating effect. The acids added directly or produced in vivo by fermentation in the milk during cheese manufacture precipitate caseins at pH 4.7, the isoelectric point. The coagulum produced thereby traps milk fat, minerals, vitamins and lactose. However, in cheeses such as Swiss, Cheddar or Provolone, precipitation occurs as a result of rennin action at pH Rennin cleaves kappa-casein into parakappa casein and glycomacropeptide. The casein micelle disintegrates, loses its hydrophilic character and calcium ions inherently present in milk precipitate alpha and beta caseins to form a coagulum. A third precipitation form, restricted mainly to Ricotta or recooked cheese, utilizes

a combination of high heat and medium acidity to partly dehydrate protein particles and precipitate the curd. Cheese whey or whole milk is acidified to a critical pH 6.0 to 5.9 with lactic acid starter, acetic acid or acid whey powder and then heated to 85 C (185 F) to form a coagulum.

# Composition of Latin American White Cheese

According to Siapantas and Kosikowski (1967) the average composition of Latin American White cheese is 50% moisture, 24.9% total protein and 19% fat. However, Davis (1976) reports that the moisture in fresh cheese ranges from 40 to 44% and is sold within a week. The drier cheese has from 35 to 39% moisture and is left to mature. Sometimes it is stored in dry salt. The very hard cheese has 27 to 32% moisture.

Composition of some Venezuelan cheeses as reported by Escoda and Hernandez (1968) is shown in Table 2.

Table 2. Chemical composition of some Venezuelan Queso Blanco cheeses.\*

	Туре	Moisture	Fat	Protein	Salt
Queso	de Ano	40.9	27.6	23.6	4.8
_	Pasteurizado de Matera	49.4 39.0	20.4 24.9	22.7 26.9	4.5 6.8

<sup>\*</sup>Escoda B. S. and E. Hernandez (1968).

In comparison with Cheddar cheese, Latin American White cheese possesses 25% fewer calories with comparable protein levels. However, Ca, P and Mg content in the white cheese is 33 to 50% lower than Cheddar cheese (Chandan et al., 1979).

Wong (1974) explained that curd made with rennet traps most of the fat and insoluble salts of the milk, whereas in the curd formed by acid precipitation the insoluble salts are rendered soluble by the acid and are largely lost in the whey. Since about one-fourth of the phosphorus is held in organic combination and since the calcium becomes soluble more rapidly than the phosphorus and other ash components, the Ca/P ratio as well as the percentage of calcium are comparatively low in cheese made under conditions of high acidity.

Ricotta, Impastata, and Latin American White cheeses contain both the casein and whey protein fractions of milk. Both beta-lactoglobulin and alpha-lactalbumin, as well as other whey proteins, are incorporated into cheese curd because they are rendered insoluble by the required combination of high acid and high temperatures (Kosikow-ski, 1977). Beta-lactoglobulin and alpha-lactalbumin fraction of the whey protein possesses the highest biological value (PER, 3.2) and are therefore considered excellent in human nutrition. Furthermore, since the whey proteins in milk are not precipitated by rennet and are generally lost in the whey, the yield of Queso Blanco

type cheese made with high heat and acid is higher than that of cheese made with rennet (Siegenthaler, 1968).

In general, the white cheese made in Latin America has good physical, chemical and nutritional properties but unsatisfactory sanitary characteristics. Boscan (1972) reports 2.7 x  $10^6$  coliforms per gram of Queso Pasteurizado and 12 x  $10^6$  coliforms per gram of Queso de Matera. This condition is caused by the poor quality of raw milk, post-pasteurization contamination and inadequate refrigeration systems.

# Keeping Quality of Latin American White Cheese

The cheese has excellent keeping quality when vacuum-packaged and kept cool (Kowsikowski, 1977). The addition of high concentrations of cheese-salt (8-25%) is a common practice to preserve the major milk constituents and to control the growth of microorganisms under tropical and primitive conditions of cheese production (Escoda, 1973).

Microbial contamination of Iranian white cheese produced from raw milk is controlled by the action of brine (Mehran et al., 1975). Siegenthaler (1968) explained a method for storing native varieties of cheese in tropical climates. The Bedouins, inhabitants of the steppes and deserts, do not salt cheese before pressing. After cutting squares of about 8 x 8 x 2 cm, the flat chunks are sprinkled with salt and piled upon each other

to let the salt penetrate. After a few hours, the cheese squares are placed in a wire basket and immersed for a few seconds in nearly boiling water containing about 10% salt. The treated chunks are kept in glass jars or earthenware containers under saturated (22%) salt solution for many months at ambient temperatures. Because of the high salt content the cheese is soaked in fresh water for a few hours before consumption.

Some of the skimmilk cheese is smoked for two or three days, which darkens the surface of the cheese and dries it somewhat in addition to giving it a smoked flavor and increased shelf-life (Wilster, 1974).

According to Davies et al. (1937) salt in cheese may influence the following: (1) the inhibition of certain types of microorganisms, (2) the activation of proteolytic enzymes of rennet, (3) the solubilization of certain proteins or protein degradation compounds, (4) the acid-base equilibria in the cheese, (5) the rate of loss of whey (moisture and soluble constituents), and (6) the proportion of bound and free water. They found that the omission of the salt resulted in 50% increase in ripening rate as measured by protein breakdown.

# Uses of Latin American White Cheese

Usually the fresh and dry "criollo" cheese is consumed grated or simply in fine crumbs, with beans, rice, tortilla (corn flat bread) and Arepas (corn bread) (Davis,

1976). Queso Llanero has a strong flavor and is used in baking or is grated over a finished dish. In Venezuela, the cheese is used to flavor sauces especially the cheese and tomato sauce called "chorreada" which accompanies boiled potatoes or meat dishes. Queso Blanco is also served with banana cake and meat to form a slightly sweet, spicy contrast (Eekhof-Stork, 1976). Panneer, an Indian form of Latin American White cheese, is quite popular in Northern India for use in curries, particularly along with spinach and peas. Peebles and Roberson (1969) and Selman and Peebles (1971) explored marketing possibilities of Queso Blanco in the United States among Latin American and other ethnic groups that desire the product for traditional recipes and snacks. Chandan and Marin (1978) attempted to increase application of Latin American White cheese by flavoring the curd with onions and garlic, caraway seeds and hot pepper or Jalapeno pepper. demonstrated the marketing possibilities of this type of cheese for the American consumer by capitalizing on the current and increasing demand for cheese. Latin American White cheese may be used as a snack, in salads, as a cooking cheese in casserole dishes, or grated for use in pizza and other foods or included as an ingredient in process cheese.

Queso Blanco may be used in process cheese manufacture in the same way as any other type of cheese (Wilster, 1974). One method successfully used in Central America

employs the following procedure: to 2.05 kg of ground white cheese are added 113.5 g of butter; 178.8 g of water and 56.8 g of emulsifier (disodium phosphate) in a steam jacketed processing kettle. After heating to 70 C in 4 min, the steam is shut off, the product is held at that temperature for 1 min and then poured into polyethylene lined boxes. A highly acceptable product was obtained by combining with aged Cheddar cheese and processed as outlined above.

Siapantas and Kosikowski (1973b) reported modification of the functionality of Latin American White cheese by coagulating the milk with rennet and creaming the curd as is conventionally practiced in Cottage cheese. Mozarellalike stringing characteristic and excellent melting properties were developed by this procedure.

# Cheese Ripening

Some cheeses are ready to eat immediately after curd is made while others need to be cured or ripened for days, weeks or months to develop proper characteritics (Van Slyke and Price, 1949). According to Escoda (1973) the ripening process of Queso Blanco cheese is a two-stage process. The first stage is relatively very fast and is produced by the microorganisms of diverse origin present in milk and capable of changing milk components. The second stage is slower and is attributed exclusively to those organisms capable of growing in a slightly acid

medium with high salt concentrations (8-25%). In both soft and semihard white cheeses the second fermentation stage does not take place. The cheese is generally consumed immediately or within 15 days after manufacture.

In the first stage of fermentation the microorganisms transform the milk components to generate certain flavor characteristics. If the microflora produces desirable flavor effects, Queso Blanco cheese manufactured with a relatively longer processing period (more than 150 min) would have a better flavor than the one made in a shorter processing time (less than 90 min). At this stage, the ripening is measured by the changes of pH. This ripening effect is slowly arrested by the increase in acidity and the decrease in moisture content.

In Latin American White cheese the salt concentration (in liquid phase) is generally from 5 to 25%. According to Foster (1957), 4% sodium chloride in liquid phase is enough to stop a ripening process.

Under the conditions in the White cheese, only certain organisms are capable of surviving and they may not always be the ones desired. Accordingly, the second stage is unreliable for consistently desirable flavor production. To obtain a good and predictable flavor, it is necessary to control the first fermentation stage.

Not much investigation has been reported on the flavor attributes and other physico-chemical ripening aspects of Latin American White cheese. However, the literature

contains a wealth of information regarding changes in protein, fat and lactose resulting in typical body, texture, and flavor of various ripened cheeses. The changes during ripening involve fermentation of lactose, partial hydrolysis of proteins, peptides and fat (Schormuller, 1968).

The ultimate quality of a given cheese depends upon both careful manufacture and proper ripening. The manufacturing procedure determines the future of the cheese by establishing the proper physical and chemical conditions under which ripening will proceed. In general, the variables are: (1) the type of microorganism, either in the milk or starter or added to the curd, (2) manufacturing methods, (3) the general curing room practices, and (4) the curing temperature and humidity. These factors combine to determine the cheese variety and the quality of the cheese within its recognized variety. (Harper and Kristoffersen, 1956).

Examples of association of kinds of cheese with ripening environment are mentioned by Van Slyke and Price (1949): mold ripened types such as Camembert, Roquefort, Gorgonzola and Blue cheese require a cool, wet environment. The types of cheese ripened with characteristic growth of yeast and bacteria on the surface (Brick, Munster, Limburger and Tilsit) also ripen optimally in a moderately cool, wet room. Swiss cheese receives its characteristic eye formation in a warm, wet room and its

final curing under conditions suitable for Cheddar.

According to Harper and Kristoffersen (1956) primary changes during ripening include transformations in carbohydrates, fat and protein. The primary phase results in the accumulation of lactic acid, fatty acids and free amino acids. Secondary changes pertain to the formation of compounds brought about by action of enzymes of ripening microorganisms on the primary compounds.

## Cheese Flavor

Flavor is a composite sensation, of which taste and odor are the important components. Those materials that are sapid are all water-soluble, while only volatile substances are odorous (Moncreff, 1966).

Collectively over 100 volatile and non-volatile potential flavor compounds have been identified in ripened cheese. With respect to their contribution to flavor, suppositions have centered primarily on acetic acid and other short chain fatty acids, amino acids, alcohols, aldehydes, ketones, esters, ammonia, amines, sulfides and mercaptans. With the exception of the short chain fatty acids, the primary ripening compounds contribute little to characteristic cheese flavor (Kristoffersen, 1973).

According to Harper (1959) a proper blend of components which can contribute to both taste and odor is essential to a balanced cheese flavor. Through distillation of an aqueous cheese suspension, he divided cheese flavor in two

groups: (1) components of the taste or non-volatile part include lactic acid, amino acids, non-volatile amines, salt and various fragments of the proteins and fats, and (2) components of the aroma or volatile part include amines, fatty acids, ketones, aldehydes, alcohols, esters and volatile sulfur compounds (hydrogen sulfide and mercaptans).

## Protein Changes

The protein during ripening of cheese is often decomposed significantly. The main change is in the casein component separated from milk either with rennet or acid (Schomuller, 1968). The protein undergoes varying degrees of hydrolysis which is dependent upon the cheese variety (Day, 1967). Cheddar cheese undergoes a series of chemical and physical changes during ripening, which cause the body of the cheese to lose its firm, tough, curdy properties and to become soft and mellow (Wong, 1974). rennet added during the cheese manufacture is capable of hydrolyzing the milk protein as far as peptides only (Berridge, 1954). Further breakdown to amino acids and smaller units is attributed to the action of proteolytic enzymes of milk and of the cheese microflora (Mabbit and Zielinska, 1956). In the hard cheeses, 25-35% of the insoluble protein of the curd may be converted into soluble protein, whereas in soft varieties as in Brie, Camembert or Limburger, all of the insoluble protein is

converted to water-soluble compounds such as peptides, amino acids and ammonia (Foster et al., 1957). Escoda and Hernandez (1968) report very low proteolysis in Latin American White cheese (1.30 to 6.82 g of free tyrosine per 100 g of protein).

A relationship between free amino acids and characteristic flavor has been observed (Harper, 1959). However, it is hard to establish that amino acids per se contribute to flavor. Perhaps proline is a specific part of Swiss flavor, and glutamic acid of Provolone, but in most varieties amino acids probably just serve as a background flavor with certain amino acids being utilized as building blocks for bacterial proteins and others as sources of energy (Harper and Kristoffersen, 1956). Cheddar cheese, the intensity of the flavor and the total concentration of free amino acids appear to be related although there is no apparent correlation with individual free amino acids (Kristoffersen and Gould, 1960). acids and peptide fractions in Blue-veined and Cheddar cheese impart "brothy" taste which constitutes an important background on which the flavor is enhanced (Day, 1967). The amino acids of the cheese protein are also of special importance in the formation of a number of fatty acids (Schormuller, 1968).

Kristoffersen (1973) reported that the development of characteristic cheese flavor appears to be determined by the ability of protein-based sulfur groups to accept hydrogen resulting from oxidative ripening processes.

Bitterness in Cheddar cheese has been ascribed to the proteolytic activity of rennet to give bitter-tasting peptides (Day, 1967). It has also been suggested that bitterness is due to the deficiency in certain strains of proteolytic enzymes capable of hydrolyzing bitter primary breakdown products of the cheese protein (Emmons et al., 1960).

The extent of the proteolytic breakdown in cheese can be judged from the production of the free amino acids (Long and Harper, 1956). In certain types of cheese such as Limburger and Camembert, proteolysis is much more extensive than in types such as Swiss (Harper and Kristoffersen, 1956). The rate, nature and extent of protein decomposition during cheese ripening is related to, and is influenced by the nature and concentration of proteolytic microbial enzymes, moisture content, the presence of lactic acid and temperature. Other factors such as pH, oxidation-reduction potential, and salt affecting enzyme activity are also involved (Wong, 1974). Escoda and Hernandez (1968) suggest that the low proteolysis in Latin American White cheese is due to the bacteriostatic action of salt on the proteolytic microorganisms.

# Carbohydrate Changes

A part of the lactose is used up through fermentation and the remainder is expelled in the whey (Day, 1967). The changes in lactose occur primarily during cheese manufacture and during the first stages of cheese ripening. Regardless of the stage at which lactic acid is formed, most of the lactose originally present disappears after 24 hr with only trace amounts of glucose and galactose detectable for the next 7 to 14 days (Wong, 1974). In the case of Camembert, Schormuller (1968) reported lactose detectable up to 14 days.

The glycolysis of lactose to lactic acid requires about 14 enzymatic steps (Harper and Kristoffersen, 1956). The rate and amount of lactic acid formation influences the quality of cheese. Lactic acid has a principal role in the development of special cheese varieties. Cheese-types such as "Cheddar" and "Provolone" require almost complete acid development before the manufacturing process is completed. In other varieties, such as Brick, the acid is developed mostly on the drainage table and sometimes not completed until after the cheese is placed in the brine tank (Harper and Kristoffersen, 1956).

The lactic acid developed represses harmful and undesirable microorganisms (coli-aerogenes, butyric acid bacteria, etc.) and promotes lactic acid bacteria which are necessary for the transformation of lactose.

Furthermore, lactic acid has a chemical and physicochemical action during cheese ripening. It regulates pH
level and the ion equilibria which is important to the
physical reaction of paracasein (Schormuller, 1968).

Propionic acid which is especially important in Emmentaler
and Tilsiter cheese is produced from lactic acid by
Propionibacterium organisms. Because of the opportunities
for reaction, various propionates (calcium, potassium, and
sodium) are formed. They are important flavor
contributors of Swiss-type cheese (Babel and Hammer, 1939).

# Lipid Changes

Ohren and Tuckey (1969) demonstrated that the lipid fraction of Cheddar-type cheese contributes more to the development of flavor than any other component (milk proteins and lactose) by making cheese from skimmilk which did not develop typical flavor.

The fat acts as a solvent for many of the flavor components and it is known to modify the flavor properties of many compounds. In addition, the fat serves as a precursor for a variety of compounds: lactones, methyl ketones, esters, alcohols, and fatty acids (Day, 1967). Some fat hydrolysis appears to be beneficial to the flavor of most ripened cheese. It is more important in cheeses like Blue and Romano than in others.

Although membrane phospholipids and their degradation products are normally found in Cheddar cheese, the final

flavor of the cheese is not affected by wide variations in phospholipid content. Membrane material in buttermilk is apparently not involved in flavor development as such. However, its removal from milk fat can affect the flavor of cheese indirectly by allowing excessive lipolysis, sometimes leading to rancidity in matured cheese (Law et al., 1973).

Fatty acids have long been recognized as important components of Blue cheese flavor. For example, the "peppery" taste has been attributed to the 4:0, 6:0 and 8:0 acids (Day, 1967). Escoda and Hernandez (1968) report moderate lipolysis (more than proteolysis) in Latin American White cheese. More relative lipolysis was observed in the case of Queso Llanero (up to 12.06% fatty acids) than in Queso Zuliano (up to 5.20% free fatty acids). little lipolysis was reported in Queso Fresco (up to 1.90% free fatty acids). These differences in lipolysis among Queso Blanco cheese varieties is due to higher contamination in the case of Oueso Llanero because of its manufacturing procedure, together with a longer period of manufacture than that of Oueso Zuliano. In the case of Queso Fresco or Queso Cocido (cooked cheese), lipase enzymes may be inactivated by the cooking step in their manufacturing procedure.

The degree to which the different fat components contribute to flavor is roughly inversely proportional to their molecular weight (Harper and Kristoffersen, 1956).

Fatty acids of intermediate chain length produced during ripening appear to be characteristic of aged Cheddar cheese (Harper, 1959).

# Lipases in Cheese Ripening

Romano and Pasta Filata Italian cheese varieties have a peppery, piquant flavor typical of free fatty acids. This flavor formerly depended upon the use of rennet paste. Presently, purified pregastric esterase preprarations are used with milk clotting enzyme extracts (Richardson et al., 1971). Rennet pastes generally consist of fourth stomach tissues and stomach contents derived from kid (goat), lamb, or calf. The stomach contents, which contain pregastric esterase and the stomach tissues are ground and prepared in a paste. The fourth stomach tissues, present in rennet pastes, are absent in purified pregastric esterase. Harper and Gould (1955) reported low lipase activity in rennet purified from rennet pastes.

# Relationship Between Different Chemical Compounds and Cheese Flavor

According to Harper (1959) the proportion of both amino acids and fatty acids are important in relation to cheese flavor. For Provolone cheese he reported that the amino acid, glutamic, and the fatty acid, butyric, must be present in certain concentrations and ratios before a

definite characteristic flavor is observed. In Swiss cheese, the amino acid, proline, and the fatty acid, propionic acid, have been related to flavor. Similar relationships may be observed in other cheeses.

According to Kristoffersen and Gould (1960) the flavor of Cheddar cheese appears to be related to the ratio of free fatty acids and hydrogen sulfide concentrations.

Cheese ripening generally is followed by increased volatile fatty acids and increased soluble nitrogen. The volatile fatty acid determination is used to monitor the development of flavor, and increased soluble nitrogen relates to the development of a mellow and waxy body. According to Dahlberg and Kosikowski (1947) the magnitude of these chemical changes is not necessarily related to the intensity of the flavor of Cheddar cheese.

# Component Balance Theory for Flavor

The available knowledge of the chemistry of cheese ripening seems to justify the conclusion that each variety of cheese, with specific flavor, body and texture characteristics is determined by a specific type of ripening process (Wong, 1974). Many flavor producing substances such as peptides, amino acids, fatty acids, aldehydes, carbonyl compounds, ketones, alcohols, esters, diacetyl, ammonia, and hydrogen sulfide have been isolated from cheese. However, pure compound substitution in flavor experiments did not identify a single compound as the

basic cause of the typical flavor. Kosikowski and Mocquot (Kosikowski, 1977) have proposed the component balance theory to explain how typical flavor originates in a ripened cheese: (1) typical cheese flavor, particularly in Cheddar and other hard rennet cheeses, apparently does not depend upon a key component, but originates from a variety of substances resulting from protein fat and lactose, (2) In the individual state, these compounds have flavors other than cheese flavor. Collectively, when in certain critical balance, the resulting cheese flavor is typical, (3) Certain individual components in this balance are more important than others. For example, some fatty acids, amino acids, amines, peptides and ketones together may develop a flavor resembling cheese but not fully. Other compounds such as esters, secondary alcohols, and acids exert some effect on typical flavor. In fact, even the bland flavor input of neutral fat and paracasein may have importance.

More recently, McGuggan, Emmons and Larmond (1979) showed that the water-soluble fraction made from Cheddar cheese exerted the greatest contribution to the flavor intensity. The authors suggest that in relation to mild cheese, flavor compounds are more easily released in the mouth by highly degraded protein of aged Cheddar cheese.

#### EXPERIMENTAL PROCEDURE

# Preparation of Latin American White Cheese

For this study, Latin American white cheese was produced from whole milk purchased from the Michigan Milk Producers Assocation. A total of eighteen 195-kg lots of raw whole milk ranging in fat content from 3.53 to 3.77% were used.

The manufacturing procedure was an adaptation of the methods given by Kosikowski (1977), Siapantas and Kosikowski (1967), and Chandan and Marin (1978). Milk was heated to 82 C (180 F) in 50-gal stainless steel cheese vats by circulating steam in the vat jacket. While heating, the milk was continuously stirred to prevent its burning on the vat surface. The heat treatment used in the preparation of the white cheese exceeds normal pasteurization temperatures. Holding times ranged during cheese making from 15 min to 25 min. Apparently, no pathogenic microorganisms could survive the heat treatment. The resulting cheese thereby should not cause any public health problem if properly handled afterwards.

Immediately after reaching 82 C (180 F), 2.39 g of U.S.P. citric acid per kg of milk was added. Prior to the addition, citric acid was placed in a stainless steel

beaker and diluted with tap water to yield a 10% solution. The citric acid solution was uniformly added to the hot milk while stirring. Milk precipitation occurred immediately. Gentle agitation was continued for three more minutes to prevent curds from matting. After settling for 15 min the curd was separated from greenish-yellow whey by draining the liquid from the vat through a metal screen at the exit gate. Curds were cooled to 32 C (90 F) by passing cold water through the vat jacket.

Since the purpose of this study was to develop flavor and texture character in Latin American white cheese, the next step was different from the original procedure used in Latin America and other tropical and subtropical countries (Sigenthaler, 1968). This difference refers to the direct treatment of the cooled curd with yogurt culture, lactic culture and lipase enzymes.

Yogurt and lactic cultures used were frozen, concentrated cultures from Chr. Hansen's Laboratory, Inc. They were kep at -27 C (-18 F) and thawed by immersing the culture can in water at room temperature. A can with 70 ml of yogurt culture concentrate was used for each 53 pound curd lot and 360 ml of Redi-set DVS lactic culture concentrate was used for each 53 pound curd lot (Sellars and Babel, 1970). The enzymes used were lipase powders marketed by Dairyland Food Laboratories, Inc. from edible, federally inspected animal tissues. Standard enzyme preparations were used; Italase C (DFL Code 01002-1), capalase

K (DFL code 01011); and capalase KL (DFL code 01012). All the enzymes were of standard strength. Levels used were those recommended by the manufacturer to obtain high flavor characteristics (Dairyland Food Laboratories, 1978). Italase C, at the rate of 0.09 g per kg of milk, is reported to give a delicate, mild, "piccante" flavor. Capalse K, 0.13 g per kg of milk, reportedly gives flavor with the following characteristics: peppery, vibrant, "goaty", sharpest "piccante". Capalase KL, at a concentration of 0.13 g per kg of milk, is described to give a peppery flavor with lingering, "piccorino" and sharp piccante characteristics.

Using the same lot of milk, appropriate amounts of cheese curd were obtained for the preparation of untreated (control) cheese as well as for cheese treated with yogurt culture, lactic culture or lipase enzymes. A total of three lots each of the curd were treated with yogurt culture, lactic culture or with lipase enzymes.

After mixing thoroughly the cheese curd with respective treatment materials at 32 C (90 F), 2% cheese salt was added. The salt was mixed for about 15 min to obtain homogenous distribution of the salt in the cheese curds. Wilson square stainless steel hoops (20 lb) were then filled with the cheese curd and pressed at 40 psi (2.8 kg/cm $^2$ ) for 20 hr at room temperature. The next morning, the cheese blocks were taken out of the hoops and vacuum packed in Cryovac Barrier bags. The cheese blocks

were kept at 7 C (45 F) and 80% humidity in the curing room and ripened up to 12 weeks.

A schematic outline for production of Latin American white cheese is shown in Figure 1.

# Analytical Procedures

The cheese samples were analyzed immediately (at zero time) for the shear force measurements, pH, fat, moisture, salt, ash, mineral, total protein, protein degradation or soluble protein, free fatty acid titer, volatile acidity and lactic bacterial count. Then at 2, 4, 8 and 12 wk of curing, they were analyzed again for shear force measurements, pH, protein degradation or soluble protein, free fatty acid titer, volatile acidity and lactic bacterial content. Except for the shear force measurements, the bacterial count and moisture determination, the ripened cheese samples were sealed in Barrier bags and held frozen at -20 C (-4 F) until the time of actual analysis.

# 1. Measurement of Shear Force

The Kramer shear press, Model SP-121MP with recording attachment was used with a 3000 pound ring and a Model CS-1 standard shear compression cell. Textural characteristics were determined by measuring degree of deformation of proving ring, resulting from the force required to compress and shear the cheese sample in the test cell. The process followed was essentially similar to that reported

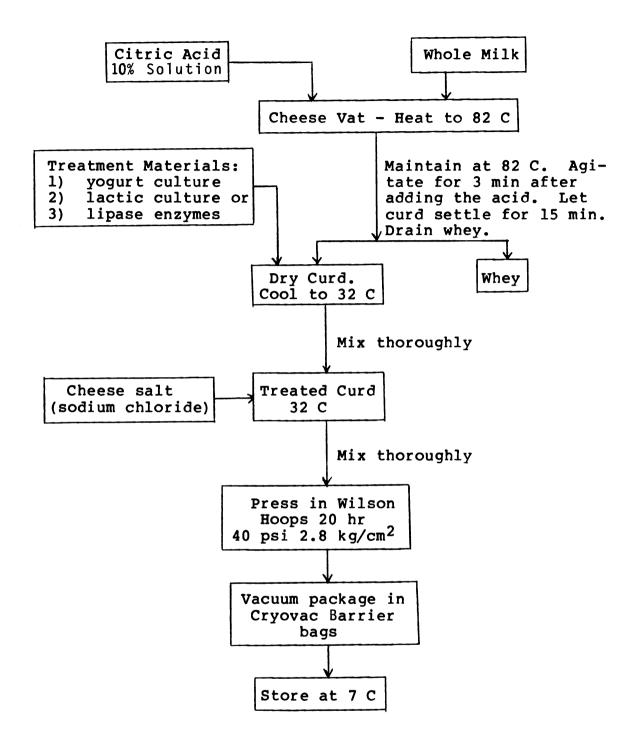


Figure 1. Flow sheet diagram for the manufacture of Latin American White cheese.

earlier by Thakur (1973) for Cheddar cheese.

- (1) An approximately 12 mm thick slice of cheese was tempered at 27 C for 5 hr, then cut to fit without forcing into the bottom of the test cell.
- (2) The element was attached to the transducer ring and the cell-box with the weighed sample covered, was placed in the proper position.
- (3) Range was set at 10.
- (4) Recorder pen was adjusted to zero.
- (5) Shear blades were passed through the sample and the resistance to shear was recorded on the chart paper.
- (6) Readings on each sample of cheese were obtained in duplicate. Maximum peak was read from the recording chart. Calculations for 1b force shear resistance per gram of sample were made using the following formula.

$$\frac{1b \text{ ring } x \quad \underline{\text{range}}}{100} \quad x \quad \underline{\text{peak height}}}{\text{weight of sample (g)}} = 1b \text{ force/g}$$

## 2. pH

The pH measurements were made with a CHEMTRIX Type 60A digital pH/mv meter equipped with a Lazar Research Labs., Inc., Model 9113 pH electrode designed for surface measurements. Before testing the pH meter was standardized with standard buffer solution, pH 4.01 and manually set for the temperature of the product. pH was determined in all samples to the nearest .01 pH unit. After each

determination, the electrode was moved to test two other areas of the same cheese. An average of the three pH readings is reported in the Results section.

## 3. Fat

Fat content in all samples was determined by the Roese-Gottlieb method with Mojonnier modification for cheese (Milk Industry Foundation, 1959). Steps followed were those described by Mojonnier Bros. Co. (1925) for determination of fat in cheese.

## First Extraction

- (1) About 1 g of cheese was weighed into extraction flask using a butter boat.
- (2) Eight milliliters of distilled water were added to the sample in the extraction flask and mixed thoroughly.
- (3) Then, 1.5 ml of ammonia were added and mixed thoroughly.
- (4) Ten milliliters of 95% alcohol were added, and the bottle was shaken for 30 sec.
- (5) Twenty five milliliters of ethyl ether were added, the bottle was covered with cork and shaken vigorously for 20 sec.
- (6) Twenty five milliliters of petroleum ether were added, the bottle covered with cork and shaken vigorously for 20 sec.

- (7) Flasks were centrifuged 30 turns, taking 30 sec.
- (8) The ether mixture containing the extracted fat was poured off into previously weighed fat dishes. Prior to use, the empty fat dishes were heated in the vacuum oven at 135 C for 5 min and then cooled in the cooling desiccator for 7 min.

# Second Extraction

- (1) This time, neither water nor ammonia was added.
- (2) Five milliliters of alcohol were added to the residue in the flask and it was shaken for 20 sec.
- (3) Then 25 ml of ethyl ether were added, the bottle covered with cork and mixed for 20 sec.
- (4) Twenty five milliliters of petroleum ether were added, the bottle covered with the cork and mixed 20 sec.
- (5) Flasks were centrifuged 30 turns, taking 30 sec.
- (6) The ether-fat solution was poured off into the same fat dish used in the first extraction. When it was necessary to raise the dividing line in the extraction flask, a proper amount of distilled water was added just before pouring. Care was taken not to pour off any of the residue below the ether solutions.
- (7) Ether was evaporated off from the fat dishes on the electric hot plate at 135 C for 5 min with not less than 20 inches of vacuum.
- (8) Dishes were cooled for 7 min in the desiccator.
- (9) Dishes were rapidly wieghed, results recorded and used for calculations of the percentage of fat.

For each sample, the test was done in duplicate and the average was reported.

# 4. Moisture

Moisture content of cheese samples was determined in duplicate by the modified Mojonnier method for moisture in cheese (Milk Industry Foundation, 1959). The procedure is as follows:

- (1) Solids dish was prepared by heating in the vaccum oven at 100 C for 10 min and then cooling in the desiccator for 5 min with the water circulating.
- (2) Approximately 0.5 g of cheese was weighed into the prepared and preweighed solids dish.
- (3) Two milliliters of hot distilled water were added and the cheese was spread using a glass rod to break up any lumps.
- (4) The dish was placed on the hot plate at 180 C, pressed with contact tool to insure uniform evaporation and heated until the first traces of brown color appeared.
- (5) The dish was transferred to the vaccum oven at 100 C for 10 min at not less than 20 inches of vacuum.
- (6) The dish was then cooled in the desiccator for 5 min with water circulating during this time.
- (7) Dish was weighed and the percentage moisture was calculated by the difference in weight of the original sample and the dried solid residue.

### 5. Salt

Salt was determined by the Marquard test (Atherton and Newlander, 1977) as follows:

- (1) Ten grams of ground cheese and 250 ml of water were brought to a boil and then let stand for 4 hr at room temperature.
- (2) After filtering through a cheese cloth, a 25-ml aliquot was titrated with .085N AgNo<sub>3</sub>(29.06 g AgNo<sub>3</sub> in a 2 liter distilled water) using 10% solution of potassium chromate as indicator. The reddish-brown color was the end point. Calculations were made using the following formula:

% NaCl in cheese = 
$$\frac{ml \ solution}{ml \ sample}$$
 weight of cheese (10 g)

#### 6. Ash

Ash in cheese samples was determined by the method described by the Association of Official Agricultural Chemists for cheese (AOAC, 1975) with some modification. First, approximately 2 g of sample were weighed into preweighed round flat-bottom metal dish of about 5 cm diameter. The dish was placed in the vacuum oven at 100 C (± .1 C) to dry to a constant weight (4 hr) under pressure less then 100 mm (4 in) of Hg. At the end of drying, the vacuum pump was stopped and air was carefully admitted into the oven. The dish was removed from oven, cooled and approximately .5 g of dry matter was weighed into a

preweighed crucible that was placed on a Corning hot plate (Model PC-35) set to 6, to ignite the dry matter and avoid spattering afterwards. When flame ceased, ignition was completed in a muffle furnace at 550 C for 20 hr. The crucible containing grey-white residue was moved from the furnace into a desiccator, cooled and weighed to determine percentage of ash.

## Minerals

The mineral content of cheese was analyzed using a direct reading spectrograph or photoelectric spectometer "Quantograph" manufactured by Applied Research Laboratories, Inc. and installed at the Horticulture Dept. of Michigan State University. The basic operational principle of this unit was described by Kenworthy (1960). Cheese samples were analyzed for P, Na, Ca, Mg, Mn, Fe, Cu, B, Zn and Al.

Sample preparation for quantometric analysis involved the ashing of .5 g of cheese solids (dry matter) overnight at 550 C. The cheese ash was then dissolved in the ashing crucible with 5 ml of HCl-Co-Li-K solution. A portion of the ash solution was transferred to a porcelain boat by use of medicine dropper. This ash solution was used directly in the excitation process by use of a revolving disc electrode. The amount transferred is not critical but should be sufficient to provide a good contact between the revolving disc electrode and the solution. Also, it

is necessary to provide enough solution to prevent complete evaporation during the excitations.

Excitation was accomplished by the use of an interrupted arc discharge that produces a uni-directional
spark-like condition. Values were read in a recording
chart to the nearest half division and from there to a
computer program designed for 0.5 g of dry matter. Output
was ppm or percentage on a dry basis. However, the
results in this study are expressed as mg/per 100 g of
cheese.

Preparation of HCL-CO-Li-K solution:

To 1 liter of distilled water were added the following reagents:

HNO <sup>3</sup>	142.6 ml	
KC1	34.07 g	99.0% assay
LiCl	38.22 g	99.0% assay
CoCl <sub>2</sub>	2.02 g	100.0% assay

# 8. Total Protein

The total protein in cheese samples was determined by the Kjeldahl method for determination of total nitrogen (AOAC, 1975) as modified by Kosikowski (1977). Process followed was:

(1) Approximately 3 g of cheese were weighed and placed in a porcelain mortar. The sample was moistened with acetic acid solution (25 ml glacial acetic acid diluted to 1 liter) and ground until smooth paste was formed.

- (2) Cheese suspension was transferred to 100 ml volumetric flask and acetic acid solution was added to the 100 ml mark. It was then warmed to 50 C (122 F) and a 25 ml aliquot was placed in 800 ml Kjeldahl flask.
- (3) Ten grams of  $K_2SO_4$ , 5 g of  $CuSO_4$   $5H_2O$  and 25 ml of conc.  $H_2SO_4$  were added to the Kjeldahl flask and digested for 3 hr. The contents of the flask were then rendered perfectly transparent.
- (4) To an Erlenmeyer receiving flask 125 ml of .1N HCl and 5 drops of methyl red indicator were added. Next, to the Kjeldahl flask 250 ml of distilled water, a small amount of 10 mesh granulated zinc and 100 ml of 45 to 50% NaOH solution were added.
- (5) The Kjeldahl flask was connected to the still, and the flask contents swirled carefully. The heat source was then turned on to collect 125-150 ml of distillate which was titrated to a light yellow color with .1N NaOH.
- (6) Simultaneously, a blank was run which consisted of no cheese but all reagents used in cheese protein analysis.

Formula used for calculations was:

For each sample, the test was done in duplicate.

## 9. Soluble Protein

Determination of soluble protein in cheese samples was done following a technique based on the method of Sharp (Dahlberg and Kosikowski, 1947). The soluble protein was extracted as follows:

- (1) Three grams (± .01) of cheese were weighed and placed in a porcelain mortar. A small amount of extracting solution at 50 C was added and the cheese was ground to a thick paste.
- (2) Diluted suspension of cheese was transferred to a 100 ml volumetric flask and more extracting solution was added to the 100 ml mark. The flask was placed in a water bath at  $50 \pm 0.1$  C and maintained at this temperature for 1 hr.
- (3) The suspension was filtered through a Whatman No. 1 fluted filter paper and 50 ml of the filtrate was placed in an 800 ml Kjeldahl flask.
- (4) Digestion and distillation steps were conducted as for total protein given above. The formula used for calculations was:

The soluble protein extraction solution was prepared as follows:

A. Stock Solution

57.5 ml glacial acetic acid

136.1 g sodium acetate (3  $H_2O$ )

47.0 g NaCl

8.9 g CaCl<sub>2</sub>

Distilled water was added to make volume to 1 liter.

B. Extraction Solution:

Dilute 250 ml stock solution to 1 liter with distilled water. All the tests were run in duplicates.

# 10. Free Fatty Acid (FFA) Titer

FFA titer was determined by the rapid silica gel method for measuring free fatty acids reported by Harper and El-Hagarawy (1956). This method is outlined below:

# Materials

- (1) A chromatographic column, 38 mm diam by 230 mm in length and with a perforated glass disc sealed into a 34/23 standard taper joint attached to a suction flask.
- (2) Silicic acid, 100 mesh powder.
- (3) 2 M phosphate buffer, pH 6.4. Stock solutions of 2M  $\rm KH_2PO_4$  (27.2 g/100 ml) and 2M  $\rm K_2HPO_4$  (34.8 g/100 ml) were prepared and mixed and the pH checked and adjusted to 6.4 using a pH meter.
- (4) Buffered silica gel slurry. A stock solution of the slurry was prepared by mixing thoroughly 50 g of dry silicic acid with 30 ml of the phosphate buffer and 200 ml of USP chloroform. The slurry was stored in a

tightly stoppered brown bottle at room temperature.

- (5) 20% H<sub>2</sub>SO<sub>4</sub>.
- (6) Eluant, five percent N-butanol in chloroform (v/v).
- (7) Titrating alkali, KOH solution, .01N in absolute alcohol.
- (8) Phenol red indicator, pH 7-8, granular dry indicator (Mallinkrodt).

# Preparation of Cheese Sample

Five grams of the cheese sample were dissolved in 5 ml of  $\rm H_2O$  and acidified to pH 1.8-2.0 (predetermined) with 0.3 ml of 20%  $\rm H_2SO_4$  to liberate free fatty acids and to stop further lypolysis. Eighteen grams of silica gel were added and the mixture was ground thoroughly. Then it was transferred to the top section of the column.

# Preparation of Chromatographic Column

The column was prepared in two sections:

(1) Bottom Section

The column was attached to a 250 ml suction flask. A filter paper disc was placed on the sintered glass bottom of the column, and 25 ml of the silica gel slurry was poured. The suction was applied quickly to form a uniform bed of the buffered silicic acid.

(2) Top Section

The acidified sample and silica gel mixture was slurried with 50 ml of 5% n-butanol in chloroform

(v/v) and transferred quantitatively on to the top of the bottom section of the column. This was repeated with two more installments of 50 ml each of the eluant. In order to expedite the extraction, suction was applied so that the eluant flow was approximately 30 ml/min. Fifteen milliliters of methanol and 100 mg of phenol red indicator were added to the eluate and titrated with 0.01N alcoholic KOH. The results were expressed as net  $\mu$ mol FFA/g cheese fat, after correction of the titer value for observed acidity of the blank sample.

# 11. Volatile Acidity

Volatile acidity was determined by the rapid direct distillation method for determining the volatile acids of cheese (Kosikowski and Dahlberg, 1946).

- (1) A 10 g sample of cheese was weighed and thoroughly ground in a porcelain mortar with warm (50-55 C) 10%  ${
  m H}_2{
  m SO}_4$ . The ground cheese mixture was washed quantitatively from the mortar into a 500 ml 2-neck distillation flask with a total of 50 ml  ${
  m H}_2{
  m SO}_4$  solution, including both grinding and washing.
- (2) Ten Hengar granules (Hengar Co., Philadelphia, PA), for smooth boiling and 35 g of MgSO<sub>4</sub> 7H<sub>2</sub>O were added to the distillation flask and exactly 250 ml of distilled water at 25 C was added. Antifoam was sprayed into flask.

- (3) Next, the flask was placed in heating mantle and connected to distilling tube. Powerstat was turned to setting 85 and flask heated rapidly to a boil.
- (4) Distillation was begun with the distillate passing through slightly moistened filter paper (Whatman No. 2) of 18.5 cm diameter and was continued until 250 ml distillate had been collected. The temperature ranged from 100 to 110 C and never over 116 C. Approaching the end of distillation (200 ml distillate collected) the powerstat setting was lowered to 75 to prevent burning of the sample. This distillate was the watersoluble portion.
- (5) This distillate was titrated with N/20 NaOH, using phenolphthalein as an indicator. The final results, however, were presented according to standard form as ml N/10 volatile acid per 100 g cheese.
- (6) Absolute alcohol (25 ml) was used to rinse the insoluble acids from the condenser through the filter paper and into a small Erlenmeyer flask. Alcohol rinsings were titrated in a manner similar to that employed with the water-soluble distillates. The sum of the titrations of the water-soluble distillates and of the alcohol rinse was considered the total volatile acidity of the cheese.

Duplicates were run on all samples.

# 12. Lactic Bacterial Count

The bacterial content of cheese samples was determined in duplicate using a lactic agar (APHA, 1976) prepared as follows:

Tryptone	20.0 g
Yeast extract	5.0 g
Gelatin	2.5 g
Glucose	5.0 g
Lactose	5.0 g
Sucrose	5.0 g
Sodium chloride	4.0 g
Sodium acetate	0.5 g
Ascorbic acid	0.5 g
Agar	15.0 g

Distilled water to make 1.0 liter

The ingredients were dissolved in distilled water by gentle heating and the medium was sterilized at 121 C for 15 min. Procedure followed was that suggested by the American Public Health Association (APHA, 1972) for the examination of dairy products.

Eleven grams of cheese was weighed and aseptically solubilized in 2% sterile sodium citrate solution by blending in a Waring blender. The required volume was pipetted to dilution bottles to obtain  $10^{-5}$ ,  $10^{-6}$ , and  $10^{-7}$  dilution. Next, 0.1 or 1 ml of the properly diluted sample was placed in a sterile petri dish (sterile -

polystyrene 100 X 15 mm) and about 10 ml of the warm (45 C) lactic agar was added, mixed and allowed to harden. The inoculated plates were incubated for 48 hr at 32 C. Plates were counted for the number of colonies and the results were expressed as an estimated number of lactic organisms/g cheese.

# 13. Organoleptic Assessment

Sensory analyses were made separately by experienced judges and by a consumer panel to evaluate flavor, body and texture of the cheese samples. Three to five experienced judges scored cheese flavor according to ADSA method for Cheddar cheese. A flavor score of 10 required no criticism. The same score card was used to evaluate body and texture. A score of 5 required no criticism on body and texture. Sample forms are shown in Figures 2A and 2B. Samples of the untreated and treated cheese were evaluated after 4, 6 and 8 wk of ripening period.

The untrained panel consisted of customers of the MSU Dairy Store and people working in the Food Science Building. A triangle test was used. A sample form is shown in Figure 3. Three samples of cheese were presented simultaneously to the tasters. Each taster was informed that two of the three samples were identical and one was different. They were asked to identify the odd sample. Besides, they were asked to indicate the sample possessing more cheese flavor, smoother texture and overall

Date	A.D.S.A.	SCORE CARD	CHEESE	D.F.I.S.A.
Contestant No.				

Perfect		Sam	ple N	lo.			Total
Score	Criticisms		<u> </u>				Grades
Flavor	Contestant →						
10	Score →	1					
	Score						
	Grade Criticism						
	Acid						
No	Bitter						
criti-	Feed			1	<b>†</b>		
cism	Fermented/fruity						
10	Flat						
	Garlic/onion			1			
	Heated			<b>†</b>	1		
	Moldy						
Normal	Rancid			1			
range	Unclean			1			
1-10	Whey taint		<del>                                     </del>				
	Sulfide			1			
	Yeasty						
			Ì	1			
Body and	Contestant						
Texture 5	Score			]	1		
	Score						
	Grade Criticism						
	Corky						
No	Crumbly						
criti-	Curdy				1		
cism	Gassy						
5	Mealy						
-	Open						
	Pasty						
	Short						
	Weak						
			1	1	1		
momar.	Total score of						
TOTAL	each sample	1	)	1		1	
	TOTAL GRADE						
	PER SAMPLE						
			I	INAI	GRA	DE	
					ÐΛ	NK	
					114	III I	

Figure 2A. Sample form of questionnaire presented to experienced judges to evaluate flavor, body and texture of Latin American White cheese.

											ce C	Ice Cream			
Milk Fl	Flavor	ы		Butter	Fla	Vor		Flavor	٤.			Body and T	Textur	ure	
Defect	လ	Δ	* ~	Defect	SD		Ы	Defect	S	Ω	Ъ	Defect	S	Ω	ы
<b>A</b> stringent	ω	_	ഹ	Acid	9	വ	4	Cooked	σ	_	ഹ	Coarse/icy	4	~	-
arn	Ŋ	က	-	Bitter	9	5	4	Lacks flav-				Crumbly	4	7	-
Bitter	2	ო	-	Briny	9	വ	4	oring	σ	∞	7	Fluffy	ო	7	H
Cooked	0	ω	9	Cheesy	က	7	-	Too high				Gummy	4	7	-
Cowy	9	ず	7	Coarse	∞	7	9	flavor	σ	ω	7	Sandy	7	-	•
Feed	0	∞	2	Cooked	თ	ω	9	Unnatural				Soggy	4	က	7
Flat	0	∞	7	Feed	0	œ	9	flavor	ω	9	4	Weak	4	7	~
Foreign	Ŋ	က	7	Fishy	7	Н	ı	High acid	4	~	Н				
Garlic/				Flat	∞	7	7	Lacks fine							
onion	വ	ო	-	Garlic/				flavor	0	œ	7				
High acid	က	Н	*	onion	ო	7	-	Lacks fresh	ہے						
Lacks fresh-	_			Malty	9	വ	4	ness	ထ	7	9				
ness	∞	7	9	Metallic	4	က	7	Metallic	9	4	7				
Malty	വ	က	7	Musty	9	Ŋ	4	Old ingre-							
alli	ß	က	_	Neutral-				dient	9	4	7				
diz	9	4	-	izer	2	4	ო	Oxidized	9	4	H				
Rancid	4	Н	ı	019				Rancid	4	7	ŧ				
Salty	ω	9	4	cream	9	വ	4	Salty	œ	7	വ				
Unclean	က	-	ı	Oxidized	4	~	-	Storage	7	9	4				
				Rancid	4	7	Н	Lacks							
				Storage	9	വ	4	sweetness	σ	∞	7				
				Tallowy	7	-	ı	Syrup							
				Unclean/				flavor	σ	7	2				
				utensil	Ŋ	4	က	Too sweet	۵ı	ω	7				
				Weedy	വ	4	m								
				Yeasty	4	က	7								

\*\* Denotes unsalable. \*S denotes slight; D denotes definite; P denotes pronounced.

Figure 2B. Suggested flavor, body and texture, and appearance and color scores with designated intensities of flavor defects for Intercollegiate Dairy Products.

	,	, ,												١															
		М		Н		٢		٢	-	7			ē	or	Д		-	-	7	_	Н		7	ı		ı	-		7
	ure			7		7		7	~	က			Cheese	Color	Ω		7	~	ო	tyg	.~		m	_		٢	~		က
1	Textur	ß		4		က		4	m	4					ഗ		4	4	4	rmi	4		4	m	ı	m	m		4
Cheese	Body and T	Defect	Firm/	rubbery	Gelatin-	sno	Mealy/	grainy	Pasty	Weak/soft			Cottage	Appearance			Free cream	Free whey	u	Lacks uniform	Matted	Shattered	curd	Slimy	ce dis	colored	O	Unnatural	color
Cottage		Д	Ŋ	Н	7	<b>L</b> )		۲	7	-	7		П	Ч	7	Н	7	-	-	7									
Cot		۵	7	വ	ω	7		က	ω	4	œ		S	4	က	സ	က	7	က	7									
		S	0	7	0	0		വ	σ	7	0	1	ω	9	വ	വ	Ŋ	4	9	4									
	Flavor	Defect	Acid	Bitter	Coarse	Feed	Fermented/	fruity	Flat	Foreign	High salt	Lacks fresh-	ness	Malty	Metallic	Musty	Oxidized	Rancid	Unclean	Yeasty	ı								
	l en	ĮΑ	7	7	7	Н	7	7	Н	7	7																		
l	tur		m	က	m	7	က	က	က	ო	ო																		
	Textur	S	4	4	4	က	4	4	4	4	4																		
Cheese	Body &	Defect	Corky	Crumbly	Curdy	Gassy	Mealy	Open	Pasty	Short	Weak																		
Cheddar		ň	Ŋ	4	9		വ		7		Н	7	က	-	4	<sub>2</sub>	Ŋ	Н											
Che		Δ	7	7	ω		9		∞		4	ω	ß	4	7	9	7	4											
	L	လ	0	0	0		ω		6		9	0	7	9	0	œ	œ	9											
	Flavor	Defect	Acid	Bitter	•	Fermented/	fruity	Flat/lacks/	flavor	Garlic/	onion	Heated	Moldy	Rancid	Sulfide	Unclean	Whey taint	Yeasty	1										

\*S denotes slight; D denotes definite; P denotes pronounced. \*\* Denotes unsalable.

a Not in contest. Lacks Uniformity will not be scored in the contest even though it
will be the official score card.

Figure 2B (cont.)

Judge				Da	te		
Sample	es Presented:	<del></del>	_			_	
ent sa	T the samples are id ample. Taste to det are, take a guess.						
Differ	rent/Odd Sample Is:						
Which	has more cheese fla	vor? .	•	odd	sample	(	)
				paired	sample	(	)
Which	has smoother textur	e?		odd	sample	(	)
				paired	sample	(	)
Which	would you prefer? .			odd	sample	(	)
				paired	sample	(	)
Other	(describe):						
					Thai	nk y	/ou.

Figure 3. Sample form of questionnaire presented to untrained panel to evaluate flavor, body and texture of Latin American White cheese.

preference. This test took place in the testing room of the Food Science Building where each taster in the booths was provided with comfortable seating, proper lighting, water for oral rinsing, and adequate space for the samples and for the score card. A total of 123 tasters took part in the three separate trials. Samples of the untreated and treated cheese were evaluated at 4, 6 and 8 wk of ripening period.

#### RESULTS AND DISCUSSION

# Composition of Latin American White Cheese

Typical composition of Latin American White cheese treated with lactic culture is compared with that of untreated control in Table 3. Similar comparative data for yogurt culture and lipase treated cheeses are shown in Tables 4 and 5. In each case, percent moisture, total solids, fat, total protein and salt content of three batches of untreated and treated cheese are shown. It was observed that the composition of all the cheese samples was similar. The moisture content ranged between 48 and 50%; fat content between 15 and 20%; total protein between 19 and 25% and salt varied between 2 and 2.5%. Extreme values such as 57% moisture and 11% fat are considered atypical, and probably resulted from errors in the manufacturing procedure. In this connection, an incomplete separation of the whey during draining could cause high levels of moisture retained in the curd. Citric acid added in excess would form a fine coagulum during manufacture leading to increased protein and fat losses in the whey.

As compared to Cheddar cheese, Latin American White cheese has approximately 15% higher moisture, comparable

Table 3. Typical composition of Latin American White cheese treated with lactic culture and compared with untreated control.\*

	Moisture	Total Solids	Fat	Total Protein	Salt
			%		
			LOT A		
Treated Control	50.31 47.93	49.69 52.07	16.54 18.11	22.91 21.78	2.66 2.34
			LOT B		
Treated Control	50.40 50.09	49.60 49.91	16.74 16.91	23.99 21.72	2.22 2.24
			LOT C		
Treated Control	45.59 50.77	54.41 49.23	17.20 23.89	22.32 19.34	1.68 1.73
			MEAN		
Treated Control	48.77 49.59	51.23 50.41	16.82 19.64	23.07 20.95	2.19 2.10

<sup>\*</sup> The data are the averages of duplicate values.

Table 4. Typical composition of Latin American White cheese treated with yogurt culture and compared with untreated control.\*

	Moisture	Total Solids	Fat	Total Protein	Salt
			%		
			LOT D		
Treated Control	53.44 55.14	46.56 44.86	17.05 15.86	21.00 20.33	2.21 2.01
			LOT E		
Treated Control	47.20 45.59	52.80 54.41	24.65 26.59	19.27 21.04	1.90 2.16
			LOT F		
Treated Control	45.40 48.70	54.60 51.30	17.10 17.00	18.00 24.35	1.24 3.15
			MEAN		
Treated Control	48.68 49.81	51.32 50.19	19.60 19.82	19.42 21.91	1.78 2.44

f \* The data are the averages of duplicate values.

Table 5. Typical composition of Latin American White cheese treated with lipase enzymes and compared with untreated control.\*

	Moisture	Total Solids	Fat	Total Protein	Salt
		· · · · · · · · · · · · · · · · · · ·	%		
			LOT G		
Capalase K Capalase KI Italase Control	47.94 48.09 50.58 48.41	52.06 51.91 49.42 51.59	19.68 18.16 18.13 19.09	24.16 24.56 22.34 23.50	2.80 2.26 1.84 2.04
			LOT H		
Capalase K Capalase KI Italase Control	57.13 56.64 50.46 50.28	42.87 43.36 49.54 49.72	11.00 11.00 15.94 15.30	23.31 23.93 26.36 26.00	2.69 2.20 2.21 1.82
			LOT I		
Capalase K Capalase KI Italase Control	43.10 46.04 48.90 45.19	56.90 53.96 51.10 54.81	15.02 12.74 16.35 12.07	27.59 26.38 28.09 26.19	2.14 2.49 2.21 1.85
			MEAN		
Capalase K Capalase KI Italase Control	49.38 50.25 49.98 47.96	50.62 49.75 50.02 52.04	15.23 13.97 16.81 15.49	25.02 24.96 25.60 25.23	2.54 2.32 2.09 1.90
Treated	49.87	50.13	15.34	25.19	2.32
Untreated	47.96	52.04	15.49	25.23	1.90

<sup>\*</sup> The data are the averages of duplicate values.

protein content and about 10 to 12% lower fat. According to Escoda and Hernandez (1968), the fat content in the whey obtained in the manufacture of Latin American White cheese is about 1.12%. In contrast, only 0.5% milk fat is lost in the whey during Cheddar cheese manufacture (Chandan, 1979b). Protein: fat ratio is comparatively higher in the white cheese. Therefore, the caloric value is 25% lower in the white cheese than in Cheddar cheese. This would form an interesting feature of the white cheese for the consumer concerned with high nutritional value and low caloric content in foods. Salt content (2-2.5%) in the experimental white cheese was relatively low compared to the cheese made in Latin America and other tropical countries where figures go up to 12.5% (Escoda, 1973). salt content in white cheese was higher compared to some ripened cheeses, such as Cheddar with 1.5%, Swiss with 1.2%, Gruyere with 1.1%, and Muenster with 1.8% (Kosikowski, 1977). The high salt content may be the cause of low proteolysis in the white cheese during ripening as observed further in this study.

The ash and mineral content (P, Na, Ca, Mg, Mn, Fe, Cu, B, Zn and Al) of the white cheese is shown in Table 6. Higher values of ash and of most elements was observed in samples of cheese treated with lipase enzymes. The commercial preparation of the enzymes used in the treatment may account for the higher mineral content. The

Ash content and elemental analysis of untreated (control) Latin American White cheese compared with treated samples.\* Table 6.

Treat- ment	Ash	ρ,	Na	ದಿ	Mg	Mn	H e	no	Д	Zn	Al
	<i>9</i> 6				mg/100	g cheese	0 S O				
Untreated (control)	4.72	356.90	665.20	341.18	20.90	.157	.315	.131	ı	2.09	.39
Lactic culture	4.99	333.61	740.5	311.16	20.40	ı	.357	.025	.025	2.45	446.
Yogurt culture	4.60	337.00	641.30	322.87	15.87	ı	.318	.222	1	2.48	ı
Capalase K	6.05	375.17	763.10	357.59	21.19	424.	.450	.228	.095	2.49	.529
Capalase KL	6.24	388.22	00.669	359.10	21.76	.136	.163	640.	.027	2.18	.136
Italase	6.11	359.06	529.40	406.56	21.12	.422	.686	.179	.143	3.27	.818

\* Values are averages of four trials.

amount of sodium in Latin American White cheese is comparable to that of Cheddar cheese. McCammon et al. (1933) reported that up to 80.4% calcium and 38.1% phosphorus of the milk was retained in American Cheddar cheese. mally from 60 to 65% of the calcium and 50 to 60% of the phosphorus of the milk is retained in Cheddar cheese. However, the retention of minerals in Cottage, Neufchatel and Cream cheese is much lower, being about 20 and 37% calcium and phosphorus, respectively. For the Latin American cheese, approximately 42% calcium and 48% phosphorus from milk was retained in the cheese. Accordingly, the amount of calcium is considerably lower than that reported for Cheddar cheese. In this context the phosphorus content is just slightly lower. The white cheese was found to contain approximately 0.35% calcium and 0.36% phosphor-As compared to Cheddar cheese, the trace elements in us. the white cheese were also lower.

In the case of sweet curd formation as in Cheddar cheese, the coagulum encloses with it most of the fat and insoluble salts of the milk. In contrast, in the curd formed by acid coagulation of milk, the insoluble salts are rendered soluble by the acid and are largely lost in the whey. Since about one-fourth of the phosphorus is held in organic combination and since the calcium becomes soluble more rapidly than the phosphorus and other ash components, the Ca/P ratio as well as the percentage of calcium are comparatively lower in cheese made under acid

conditions (Wong, 1974).

### Microbial Content of Latin American White Cheese

Number of lactic organisms observed in Latin American White cheese using lactic agar are shown in Tables 7, 8 and 9. In all cases, the values obtained for the same cheese among different batches differed considerably. In most cases, the number of microorganisms reached a maximum in the first two weeks of ripening. Then a decrease in the number was observed between 4 and 8 weeks with slight increase at 12 weeks of curing.

Most bacteria disappear during ripening of a cheese except the more resistant ones which survive during this period. The lactic acid streptococci succumb almost totally in a Cheddar cheese within a few weeks but are replaced by lactobacilli, whereas the enterococci resist total destruction for much longer periods. Other ripening bacteria exist in small numbers in the young cheese, which then grow luxuriantly by symbiotic relationship (Kosikowski, 1977). Such ecological conditions may be prevalent in Latin American White cheese.

According to Harper and Kristoffersen (1956), the microorganisms responsible for the secondary changes in milk constituents during ripening of cheese begin to become more dominant as the lactic bacteria, responsible for the development of acid, begin to decrease in number.

Table 7. Number of lactic organisms observed during ripening of Latin American White cheese treated with lactic culture as compared to untreated control.\*

		Time of	Ripening	(weeks)	
	0	2	4	8	12
		count	in milli	ons/g che	eese
			LOT A		
Treated	1.40	90.00	29.00	28.00	30.00
Control	1.20	16.00	0.65	6.00	30.00
			LOT B		
Mmaak ad	г оо	23.00	7 10	0 01	7 00
Treated Control	5.00 0.04	21.00 3.90	7.10 0.40	0.84 0.51	1.00 2.10
		3,7,0		0.72	
			LOT C		
			пот с		
Treated	TNTC	3.60	3.30	2.70	3.00
Control	0.09	2.20	5.70	2.30	2.00
			MEAN		
Treated	_	38.20	13.13	10.51	11.33
Control	0.44	7.36	2.25	2.94	11.36

<sup>\*</sup> Data are averages of two values.

Table 8. Number of lactic organisms observed during ripening of Latin American White cheese treated with yogurt culture as compared to untreated control.\*

		Time	of Ripenia	ng (weeks	s)
	0	2	4	8	12
		count	in millio	ons/g che	eese
			LOT D		
Treated Control	0.77 0.28	TNTC TNTC	130.00 26.00	20.00	24.00 25.00
			LOT E		
Treated Control	3.10 2.20	TNTC TNTC	14.00	12.00 19.00	50.00 50.00
			LOT F		
Treated Control	1.80 0.10	20.00	30.00 0.15	13.00 16.00	14.00 15.00
			MEAN		
Treated Control	1.89 0.86	<u>-</u>	58.00 12.05	15.00 12.66	29.33 30.00

<sup>\*</sup> Data are average of two values.

Table 9. Number of lactic organisms observed during ripening of Latin American White cheese treated with lipase enzymes as compared to untreated control.\*

		Time of	Ripening	(weeks)	
	0	2	4	8	12
		count in	millions/	g cheese	
			LOT G		
Treated	0.72	11.80	0.40	0.73	40.13
Control	0.45	20.00	1.30	1.00	0.09
			LOT H		
Treated	0.25	10.33	6.96	78.66	21.53
Control	1.60	0.61	0.48	0.39	0.60
			LOT I		
Treated	1.19	7.93	9.83	15.63	8.60
Control	0.42	4.00	75.00	8.00	4.10
			MEAN		
mnoatod	0.72	10.02	F 72	21 67	22 112
Treated Control	0.72 0.82	8.20	5.73 25.59	31.67 3.13	23.42 1.60
				- •	

<sup>\*</sup> Data are averages of 6 values obtained with three different enzymes.

In addition to lactic agar plates, the cheese treated with lactic and yogurt culture was plated on Standard Plate Count agar. At zero and two weeks of age, SPC counts were considerably higher than those obtained using lactic agar, indicating the presence of organisms other than the lactic organisms in the cheese. The non-lactic organisms may be responsible, in part, for the physical and chemical changes taking place during ripening.

The growth pattern of organisms in the treated cheese was similar to that in the untreated control. Once again, there was an indication of the presence of microflora other than the added culture affecting flavor development in the cheese.

## Changes in Protein During Ripening of Latin American White Cheese

During ripening, protein is one of the milk constituents that undergoes a series of physical and chemical changes. Proteolysis is governed by the starter culture enzymes, and in part by the coagulating enzyme, rennet, used in the formation of the curd. Practically all of the nitrogenous constituents of young cheese exist as waterinsoluble protein. As ripening progresses, part or all of the protein is hydrolyzed enzymatically to simpler compounds that are soluble in water (Foster et al., 1957).

The general course of proteolysis is:

$$+H_2O$$
  $+H_2O$   $+H_2O$   $+H_2O$   $+H_2O$  Protein  $\longrightarrow$  Proteoses  $\longrightarrow$  Peptones  $\longrightarrow$  Peptides  $\longrightarrow$  Amino Acids (insoluble) (  $\leftarrow$ ----- water soluble ------ )

The transformation of protein in Latin American White cheese was determined by periodic measurements of non-protein nitrogen in water extracts of cheese at different ages. The experimental data are presented in Tables 10, 11 and 12 for cheese treated with lactic culture, yogurt culture and lipase enzymes, respectively. The data are plotted in Figures 4, 5 and 6 for discernibility of trend in each case.

In the case of cheese treated with lactic culture, there is a slight but progressive protein breakdown (from 0.83 g water soluble protein to 1.11 g per 100 g cheese during 12 wk of ripening). This change corresponds to 4.8% of the original protein. In the case of cheese treated with lipase enzymes, as expected, no increase in soluble nitrogen was observed. Cheese treated with yogurt culture exhibited the highest proteolysis. At zero time, 0.94% of the protein was water soluble and the value increased to 1.18% after 8 wk of ripening. This corresponds to 6.08% of the original protein. Lactobacilli are reported to bring about more rapid and extensive protein hydrolysis as compared to streptococci (Marth, 1963; Searles et al., 1970).

According to Foster et al. (1957), conditions that

Table 10. Changes in water soluble protein compounds during ripening of Latin American White cheese treated with lactic culture.\*

***		Time of	Ripening	(weeks)	
	0	2	4	8	12
			LOT A		
Treated	0.78	0.84	0.95	0.96	1.00
Control	0.94	1.04	1.02	0.96	1.04
			LOT B		
Mmoot od	0.80	0 01		0 06	1 00
Treated Control	0.82 0.88	0.91 0.80	0.99 0.92	0.96 0.95	1.02 0.86
			LOT C		
Treated	0.89	1.25	1.25	1.18	1.32
Control	0.68	0.73	0.72	0.77	0.79
			MEAN		
	0.0-				
Treated Control	0.83 0.83	1.00 0.85	1.06 0.89	1.03 0.89	1.11 0.90
	-	-	-	-	-

<sup>\*</sup> g water soluble protein per 100 g of cheese. The data are the averages of duplicate tests.

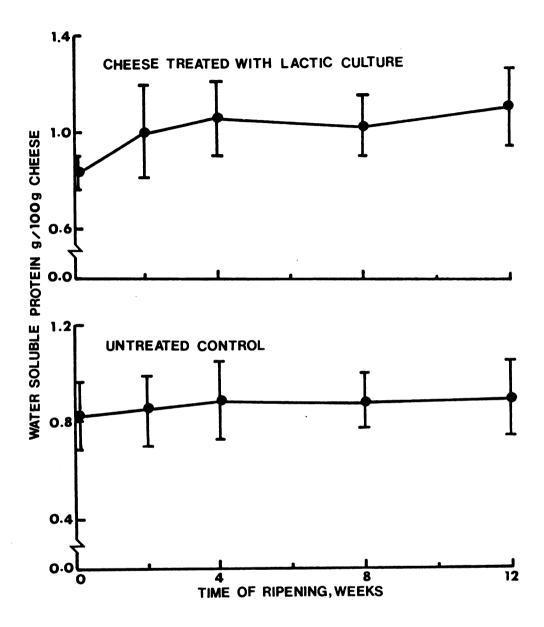


Figure 4. Changes in water soluble protein compounds during ripening of Latin American White cheese treated with lactic culture.

Mean and standard deviations are shown in each case.

Table 11. Changes in water soluble protein compounds during ripening of Latin American White cheese treated with yogurt culture.\*

	<del></del>				
			Ripening		
	0	2	4	8	12
			LOT D		
m , ,	0.00	0.00	0.06	0.07	
Treated Control	0.88 0.85	0.92 0.94	0.96 0.94	0.97 0.97	0.98 1.05
00110101	0.07	0.54	0.74	0.91	1.07
			I OM E		
			LOT E		
Treated	0.97	1.02	1.25	1.32	1.30
Control	1.05	1.06	1.19	1.17	1.28
			LOT F		
Treated	0.97	0.96	1.02	1.26	1.22
Control	0.87	1.04	0.98	1.04	1.06
			MEAN		
Treated	0.94	0.96	1.08	1.18	1.11
Control	0.92	1.01	1.03	1.06	1.13

<sup>\*</sup> g soluble protein per 100 g cheese. The data are the averages of duplicate tests.

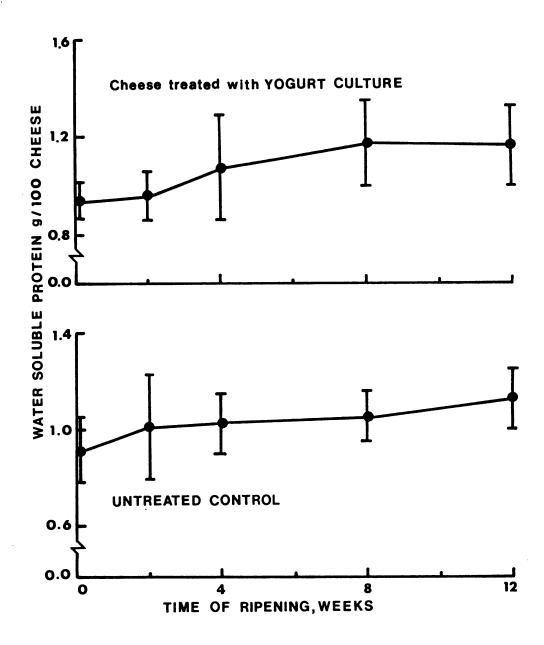


Figure 5. Changes in water soluble protein compounds during ripening of Latin American White cheese treated with yogurt culture.

Table 12. Changes in water soluble protein compounds during ripening of Latin American White cheese treated with lipases.\*

				Ripening	(weeks)	
		0	2	4	8	12
				LOT G		
Capalase Capalase	K KL	0.74 0.78	0.88	0.73 0.75	0.40 0.87	0.44 0.67
Italase Control		0.92	0.82	0.49	0.56 1.05	0.52
33			1.00	1001	1.00	1.10
				LOT H		
-	K	0.71	0.90	0.66	0.89	0.88
Capalase Italase	KL	0.88	0.88	0.56 0.83	0.97 0.99	0.98
Control		0.82	0.82	0.94	0.98	0.83
				LOT I		
Capalase		0.73	0.78	0.69	0.78	0.81
Italase	KL	0.79 0.86	0.87 0.88	0.80 0.84	0.84 0.91	0.95 0.86
Control		0.80	0.91	0.91	0.95	0.97
				MEAN		
-	K	0.72	0.85	0.69	0.69	0.71
Capalase Italase	KL	0.81 0.89	0.88 0.86	0.70 0.72	0.89 0.82	0.86 0.73
Control		0.84	0.93	0.97	0.99	0.97
Treated		0.81	0.86	0.70	0.80	0.77
Untreated	l	0.84	0.93	0.97	0.99	0.97
	·					

<sup>\*</sup> g water soluble protein per 100 g cheese. The data are the average of duplicate tests.

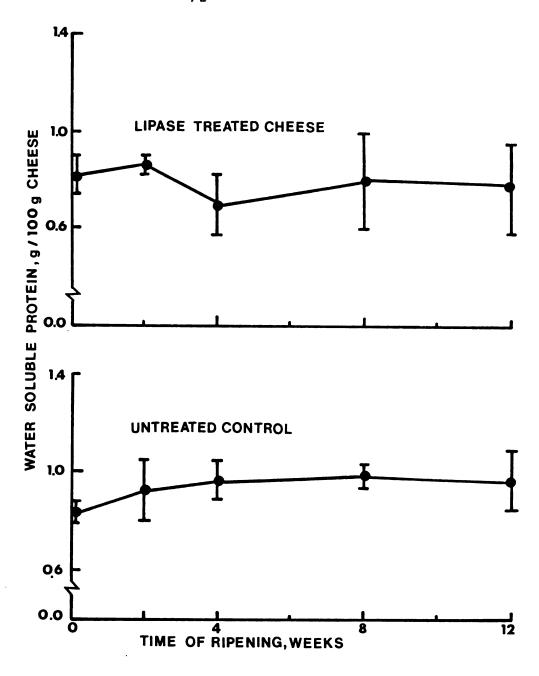


Figure 6. Changes in water soluble protein compounds during ripening of Latin American White cheese treated with lipase.

increase the rate and extent of proteolysis are: (1) higher ripening temperature, (2) concentration of rennet, (3) higher moisture, (4) salt, and (5) pH. As stated before, Latin American White cheese was made by acid and heat precipitation of the milk proteins. Thus, no rennet was present to effect any proteolytic action. The increase of water soluble protein in cheese treated with yogurt or lactic culture may be attributed mainly to the action of these organisms. This increase is relatively low compared to Cheddar cheese, where the soluble protein concentration increases from 2% at 4 wk of ripening to 12% after a period of 1 year (Kosikowski, 1977).

Yamamoto and Yoshitake (1962) found lower levels of amino acids in Cheddar cheese made without starter cultures. This effect is similar to the low values of protein breakdown observed in untreated control and the white cheese treated with lipase enzymes. Another factor that could contribute to the lower proteolysis in the white cheese is its relatively high salt content (2-2.5%). Certain microorganisms, such as proteolytic bacteria, are particularly sensitive to NaCl in the concentration found in the cheese. Escoda and Hernandez (1968) reported NaCl concentration as the main cause of low proteolysis in Latin American White cheese. Davies et al. (1937) reported that the absence of salt may increase the non-protein nitrogen in Cheddar cheese by nearly 50%.

During manufacture of renneted curd, the casein alters

its molecular configuration. Initially, during renneting, the casein changes from spherical micelles to a filament structure. During ripening this structure is transformed to a three-dimensional fibrous network (Sherman, 1976). It is the structure and arrangement of the protein molecules which are largely responsible for the textural characteristics of cheese. Curd formed by the action of acid alone, as the pH approaches that of the isoelectric point of casein (4.6), is not elastic but gelatinous and fragile, and tends to shatter more and contract less than that formed by rennet (Wong, 1974). This may explain why Latin American White cheese was considered as mealy, open, crumbly and short in body by experienced judges. Another dimension added to the texture of the white cheese is the presence of whey proteins along with casein.

Foster et al. (1957) states that some of the products of protein hydrolysis contribute to flavor, and that peptones, in general, are bitter and they may account, in part, for the background flavor. In the sensory evaluation of the cheese, no bitter flavor was indicated. This observation may be related to extremely low level of protein breakdown. Further determination of the extent of proteolysis and specific compounds found in white cheese would help to determine the flavor and textural characteristics of the cheese.

#### Evaluation of Body by Kramer Shear Press

Kramer shear press was used to evaluate body and textural chracteristics of the experimental cheese, by measuring degree of deformation of proving ring, resulting from force required to compress and shear the sample in the test cell. Compression-shearing of standard cell simulates action of teeth chewing food.

The data presented in Tables 13, 14 and 15, and Figures 7, 8 and 9 show in general, a slight increase in shear-force value in all treated and untreated cheese as a function of ripening period. In the case of cheese treated with lactic culture and lipase enzymes, the increase of firmness measured as shear-force value was 22 and 14%, respectively, after 12 wk of ripening. Latin American cheese treated with yogurt culture showed the lowest increase in firmness (7.35%) in 12 wk. As shown earlier, the cheese treated with yogurt culture displayed the highest protein hydrolysis measured as water soluble protein. Thus, the shear press measurements support the fact that solubilization of protein is an important factor responsible for the softness of the cheese.

There was also a slight increase in firmness during ripening of untreated control cheese. For all cheeses, however, the changes in hardness were small. As compared to Cheddar cheese, the values for the white cheese were comparable indicating similar hardness for these two types of cheeses. It is interesting to note that from the

Table 13. Changes in shear force during ripening in Latin American White cheese treated with lactic culture.\*

		Time of R	ipening (	weeks)	
	0	2	4	8	12
			LOT A		
Treated	2.16	2.20	2.70	2.76	3.18
Control	2.58	2.42	2.38	2.58	3.11
			T OM D		
			LOT B		
Treated	2.77	2.91	3.23	2.53	3.74
Control	2.49	2.87	3.40	3.06	3.26
			LOT C		
		_			
Treated Control	2.87 2.49	3.18 2.95	2.48 3.00	3.36 3.06	3.04 3.24
001101 01	2.17	2.77	J•00	J. 00	J•21
			MEAN		
Treated	2.60	2.76	2.80	2.88	2 22
Control	2.52	2.74	2.93	2.90	3.32 3.20

<sup>\*</sup> lb force/g cheese. Data are the averages of duplicate values.

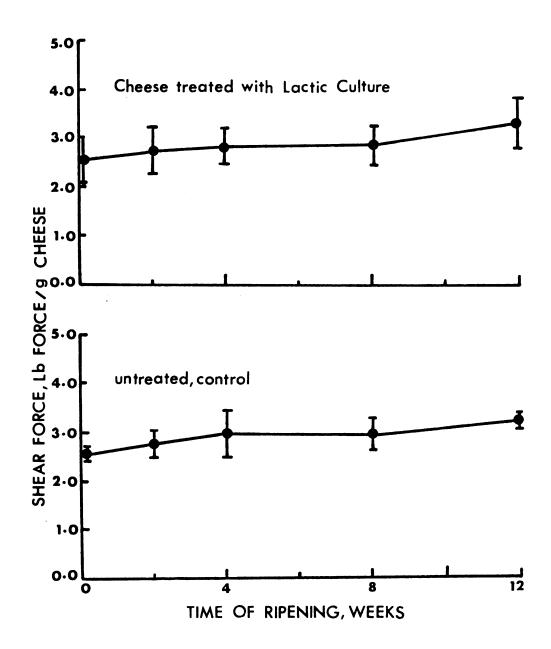


Figure 7. Changes in shear force during ripening of Latin American White cheese treated with lactic culture.

Table 14. Changes in shear force during ripening in Latin American White cheese treated with yogurt culture.\*

		Time of	Ripening		
·	0	2	4	8	12
			LOT D		
Treated	2.15	2.23	2.45	3.29	2.42
Control	2.49	2.78	3.40	3.06	3.26
			LOT E		
			пот п		
Treated Control	1.48 1.59	1.82 2.06	2.05	1.93	1.72 1.85
CONTROL	1.09	2.00	2.15	1.57	1.05
			LOT F		
Treated Control	2.78 1.69	3.03 2.26	3.14 2.63	2.76 1.89	2.80 2.93
00110101	1.09	2.20	2.05	1.09	2.73
			MEAN		
Treated Control	2.14 1.92	2.36 2.37	2.55 2.73	2.66 2.17	2.31 2.68
00110101	1.76	L • J i	- • 1 )	C • T	2.00

<sup>\*</sup> lb force/g cheese. Data are the average of duplicate values.

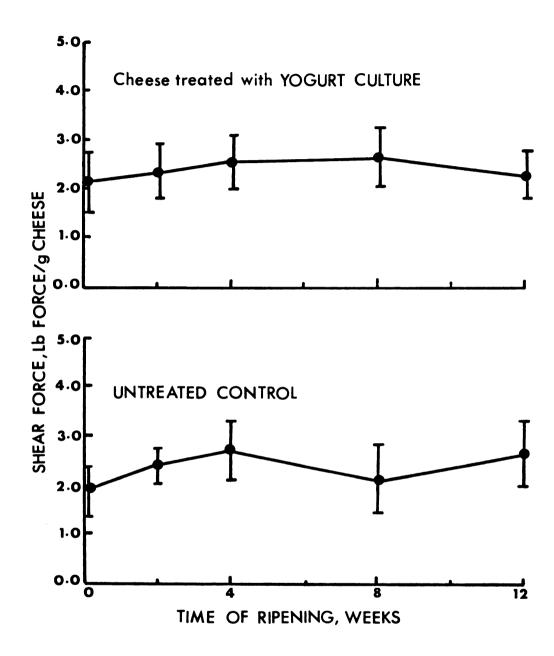


Figure 8. Changes in shear force during ripening of Latin American White cheese treated with yogurt culture.

Table 15. Changes in shear force during ripening in Latin American White cheese treated with lipases.\*

		0	Time of	Ripening 4	(weeks)	12
		<del></del>				
				LOT G		
•	K KL	3.37 2.97 2.52 2.77	3.18 3.03 2.70 2.74	2.89 2.82 2.07 2.69	4.48 3.75 2.82 3.60	3.56 3.65 3.12 3.81
				LOT H		
Capalase R Capalase R Italase Control	K KL	2.29 2.30 3.29 2.49	3.45 2.41 3.12 3.03	3.51 3.08 3.14 3.40	3.34 3.42 3.31 2.53	3.34 3.35 3.30 3.74
				LOT I		
Capalase P Capalase P Italase Control		2.60 3.09 2.59 2.49	2.47 3.07 2.76 2.95	2.46 3.00 2.86 3.00	2.85 2.93 3.42 3.06	3.12 3.82 2.92 3.24
				MEAN		
•	K KL	2.75 2.78 2.80 2.58	3.03 2.84 2.86 2.90	3.12 2.97 2.69 3.03	3.52 3.36 3.18 3.06	3.34 3.27 3.11 3.26
Treated		2.78	2.91	2.93	3.35	3.24
Untreated		2.58	2.90	3.03	3.06	3.26

<sup>\* 1</sup>b force/g cheese. Data are the averages of duplicate values.

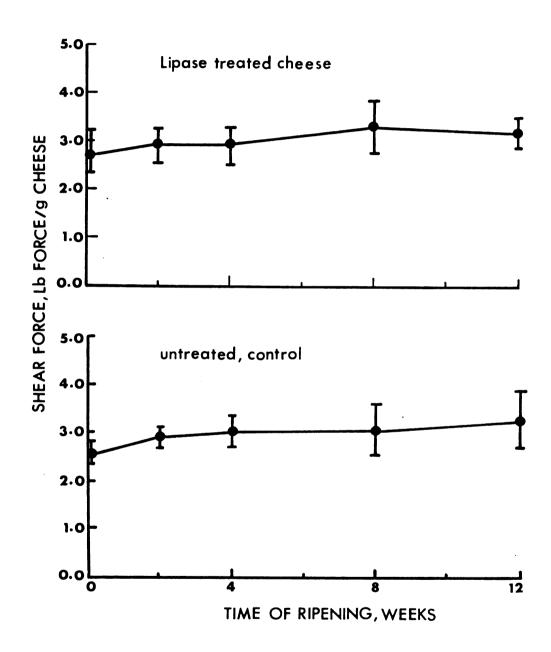


Figure 9. Changes in shear force during ripening of Latin American White cheese treated with lipase.

standpoint of texture hardness, higher moisture content of the white cheese is counterbalanced by higher fat content of Cheddar cheese.

### Changes in pH During Ripening

Normally there is a gradual increase in pH of most cheeses during ripening. This is caused by the destruction of the lactic acid, formation of non-acidic transformation products and appearance of weaker or less dissociated acids, like acetic and carbonic. The liberation of alkaline products of protein decomposition contribute further to pH rise (Wong, 1974).

The pH value in Cheddar cheese decreases to between 4.95 and 5.3; mostly between 5.0 and 5.2 during the first few days. It increases only slightly for a few months thereafter, finally increasing more rapidly to approximately 5.3 to 5.5 in 1 yr. These figures are typical of the trend that is observed in normal cheese, but a product of atypical composition may show widely different values (Foster et al., 1957). In the case of Latin American White cheese, as is seen in Tables 16, 17 and 18, and Figures 10, 11 and 12, the pH of the cheese decreased as a function of time and ripening. Although rate of decrease differed somewhat for the individual cheeses, the mean values were within pH 4.9 and 5.8. The pH of cheese treated with lactic culture was generally lower. The gradual decrease in pH in the white cheese may be caused

Table 16. Changes in pH during ripening of Latin American White cheese treated with lactic culture.\*

		Time of	Ripening	(weeks)	
	0	2	4	8	12
			LOT A		
Treated	5.20	5.38	4.79	4.69	4.86
Control	5.66	5.33	5.26	5.16	5.08
			LOT B		
Treated	5.20	5.11	5.10	5.08	5.05
Control	5.53	5.41	5.44	5.30	5.37
			LOT C		
		li aa		h. 0-	1
Treated Control	5.23 5.22	4.93 4.94	4.83 4.83	4.81 4.99	4.90 5.03
			MEAN		
Treated	5.21	5.14	4.91	4.86	4.94
Control	5.47	5.23	5.18	5.15	5.16

<sup>\*</sup> Data are the averages of triplicate samples.

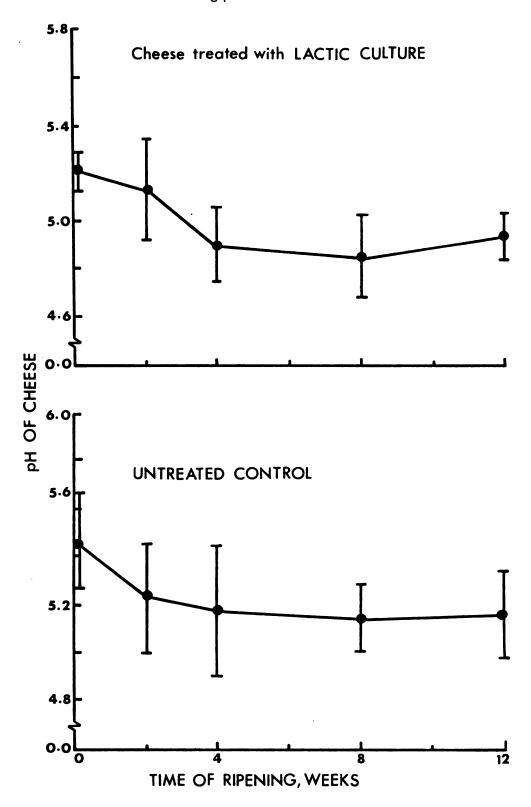


Figure 10. Changes in pH during ripening of Latin American White cheese treated with lactic culture.

Table 17. Changes in pH during ripening of Latin American White cheese treated with yogurt culture.\*

		Time o	f Ripenin	g (weeks	)
	0	2	4	8	12
			LOT D		
Treated	5.47	5.29	5.06	4.77	4.78
Control	5.61	5.54	5.00	4.96	4.72
			LOT E		
Treated	5.60	5.48	5.46	5.27	5.15
Control	6.05	5.40	5.40	5.47	5.32
			T O		
			LOT F		
Treated	5.91	5.83	5.47	5.40	5.25
Control	5.63	5.54	5.63	5.34	5.22
			MEAN		
Treated Control	5.66 5.76	5.53 5.49	5.33 5.34	5.15 5.26	5.06 5.09
OOHOLOL	J•10	ノ・マフ	۳٠ ک	٧٠٤٥	J• U J

<sup>\*</sup>Data are the averages of triplicate values.

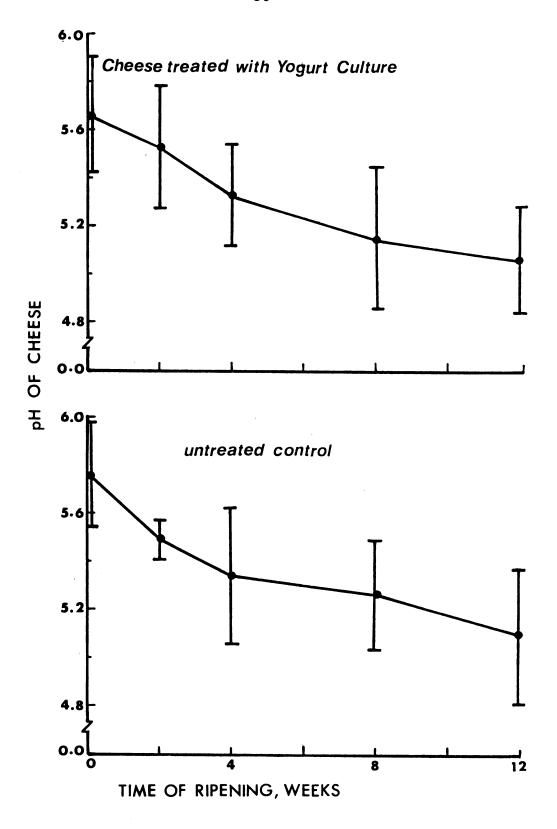


Figure 11. Changes in pH during ripening of Latin American White cheese treated with yogurt culture.

Table 18. Changes in pH during ripening of Latin American White cheese treated with lipase enzymes.\*

Treatment	Time of Ripening (weeks)					
	0	2	4	8	12	
		LOT	G			
Capalase K	5.28	5.36	5.04	5.07	5.05	
Capalase KL Italase	5.44 5.35	5.01 5.17	5.16 5.19	5.01 4.93	5.00 5.17	
Control	5.22	5.17	5.18	4.94	5.06	
		LOT	Н			
Capalase K	5.77	5.55	5.33	5.12	5.13	
Capalase KL	6.03	5.53	5.54	5.08	5.40	
Italase Control	5.21 5.68	5.54 5.39	5.44 5.41	5.10 5.36	5.19 5.54	
		LOT	I			
Capalase K	6.48	5.55	5.66	5.30	5.31	
Capalase KL Italase	5.80 6.22	5.58 5.64	5.65 5.53	5.61 5.73	5.38 5.37	
Control	5.67	5.58	5.50	5.62	5.51	
		MEAI	N			
Canalaga V	r 01ı			<b>5.16</b>	г 16	
Capalase K Capalase KL	5.84 5.76	5.49 5.37	5.34 5.45	5.16 5.23	5.16 5.26	
Italase Control	5.60 5.53	5.45 5.40	5.39 5.88	5.25 5.31	5.24 5.37	
Treated	5.73	5.44	5.39	5.22	5.22	
		-		-	-	
Untreated	5.53	5.38	5.38	5.31	5.37	

<sup>\*</sup> Data are the averages of triplicate values.

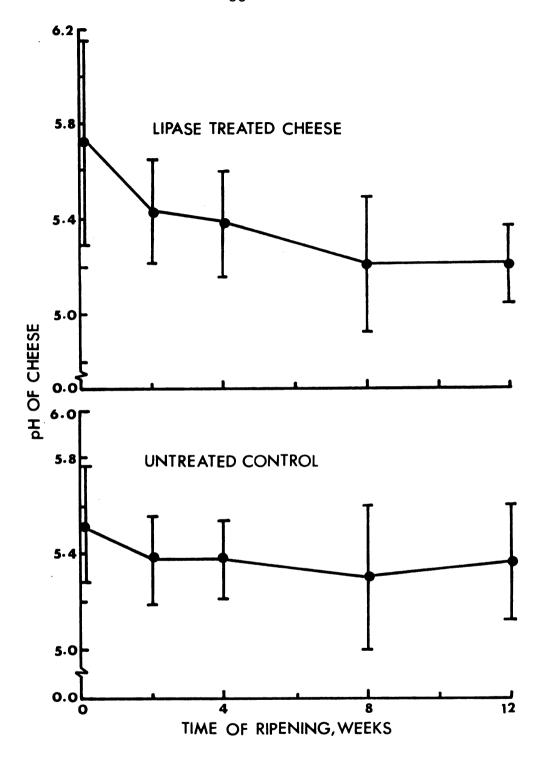


Figure 12. Changes in pH during ripening of Latin American White cheese treated with lipase enzymes.

by little or no metabolism of lactic acid and by limited production of alkaline products related to low protein hydrolysis. For the cheese treated with yogurt culture the mean decrease in pH during ripening (5.66 to 5.06) was less than that of the cheese treated with lactic culture (5.21 to 4.94). It was reported earlier that the cheese treated with yogurt culture exhibited a higher rate of proteolysis.

In the case of cheese treated with lipase enzymes, the decrease in pH value (5.73 to 5.22) could be accounted for by the production of free fatty acids (FFA). This decrease in pH became steady between 8 to 12 wk of ripening of lipase treated cheese.

# Lipolytic Changes in Latin American White Cheese

Liberation of Free Fatty Acids.

Lipases occur principally from three sources. Normal milk contains lipases. Microorganisms involved in the ripening process produce intracellular lipases and rennet pastes for Italian cheese manufacture exhibit lipolytic action. Normal rennet extract has little or no lipolytic activity. The lipases liberate fatty acids during ripening and thus increase the volatile acidity and enhance flavor (Wong, 1974). Lipolysis in Latin American White cheese was determined by extraction and titration of

the free fatty acids (FFA) and it was reported as FFA titer or  $\mu$ mol of FFA per gram of fat in cheese. Extraction on silicic acid columns was achieved by progressively eluting the adsorbed lipids with a solvent mixture of chloroform and butanol. Results are shown in Tables 19, 20 and 21, and Figures 13, 14 and 15.

The data showed there was a marked difference in the increase of free fatty acid titer of the cheese treated with lipase enzymes (58% increase) and the ones treated with lactic and yogurt culture. After 8 wk of ripening, the mean FFA titer for the cheese treated with lipase enzymes was 78.89  $\mu mol$  FFA/g fat in cheese. For the cheese treated with lactic culture the value was 29.52  $\mu$  mol FFA/g fat and it was 27.54  $\mu mol$  FFA/g in cheese treated with yogurt culture. It is interesting to note that the cheese treated with lipase enzymes had much higher FFA titer at zero time of ripening. During manufacture, cheese was left overnight in the press at room temperature. It is believed that a lipolysis of fat occurred during pressing.

The lipase enzymes were powdered enzyme preparations normally used for flavor development in Italian-type cheeses (Dairyland Food Laboratories, 1977). They are prepared from edible animal tissues.

In the manufacture of Italian hard grating cheeses, lipases are added via the rennet paste or as enzyme preparations from the base of the tongue of a lamb, kid or

Table 19. Effect of lactic culture treatment on free fatty acid content of Latin American White cheese during ripening.\*

0	Time of R	ipening (	weeks)	12
		LOT A		
14.21	22.63	32.08	37.36	35.91 25.40
14.94	22.03	20.29	21.03	27.40
		LOT B		
17.21	25.36	24.24	25.00	23.66
16.79	20.71	19.59	21.72	23.85
		LOT C		
16.65	17.50	22.58	26.20	19.36
22.85	24.21	26.38	17.00	17.00
		MEAN		
16.02	21.83	26.30	29.52	26.31
18.06	22.52	22.09	22.18	22.08
	14.21 14.54 17.21 16.79	0 2  14.21 22.63 14.54 22.63  17.21 25.36 16.79 20.71  16.65 17.50 22.85 24.21	LOT A  14.21	LOT A  14.21 22.63 32.08 37.36 14.54 22.63 20.29 27.83  LOT B  17.21 25.36 24.24 25.00 16.79 20.71 19.59 21.72  LOT C  16.65 17.50 22.58 26.20 22.85 24.21 26.38 17.00  MEAN  16.02 21.83 26.30 29.52

<sup>\*</sup>  $\mu$ mol FFA per g fat in cheese. The data are the averages of duplicate tests.

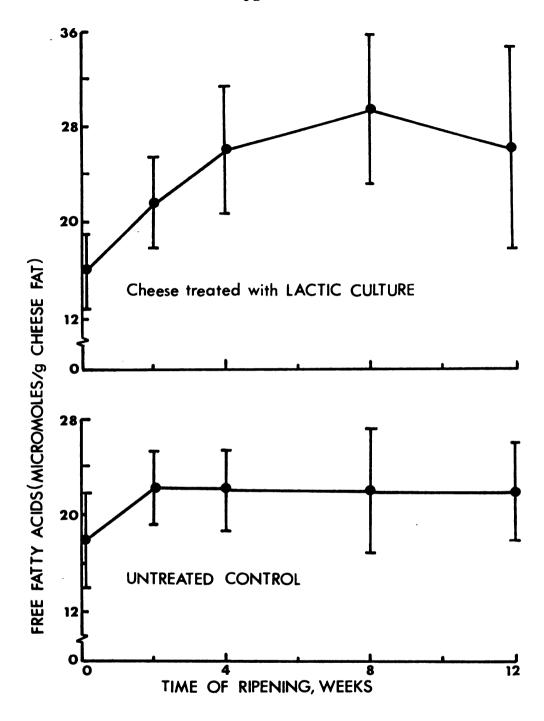


Figure 13. Effect of lactic culture treatment on free fatty acid content of Latin American White cheese during ripening.

Table 20. Effect of yogurt culture treatment on free fatty acid content of Latin American White cheese during ripening.\*

		Time of	Ripening	(weeks)	
	0	2	4	8	12
			LOT D		
Treated	16.79	22.45	28.37	33.71	27.76
Control	23.50	27.13	23.67	29.21	21.74
			LOT E		
			LOIE		
Treated	20.34	23.10	27.93	21.98	21.56
Control	14.91	17.11	18.65	19.03	19.11
			LOT F		
			LOI F		
Treated	12.67	17.04	17.16	26.93	10.97
Control	8.06	12.97	20.82	21.33	15.76
			MEAN		
			MEAN		
Treated	16.60	20.86	24.49	27.54	20.10
Control	15.49	19.07	21.05	23.19	18.87

<sup>\*</sup> µmol FFA per g fat in cheese. The data are the averages of duplicate tests.

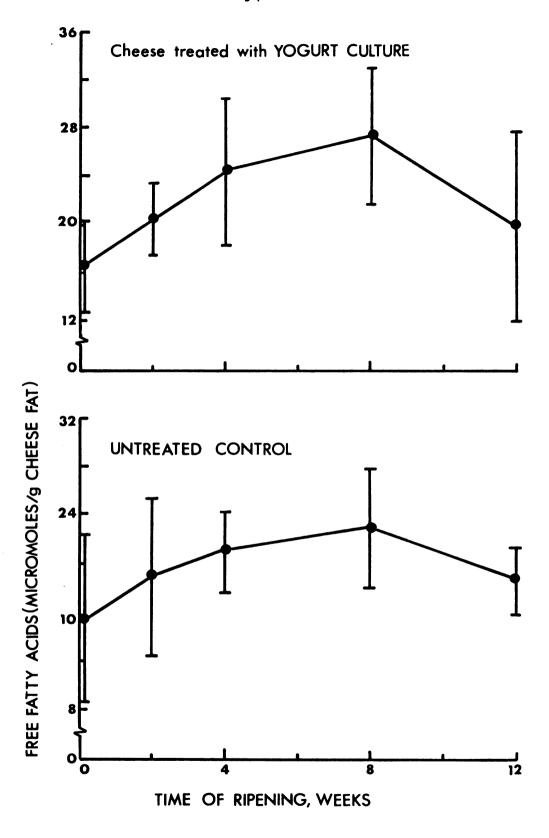


Figure 14. Effect of yogurt culture treatment on free fatty acid content of Latin American White cheese during ripening.

Table 21. Effect of lipase treatment on the free fatty acid content of Latin American cheese during ripening.\*

			of Ripening		
	0	2	4	8	12
			LOT G		
Capalase K Capalase KL Italase Control	25.97 30.43 27.07 25.19	37.28 32.88 29.17 18.82	44.74 71.05 38.80 20.14	85.16 84.07 87.92 24.92	62.74 105.88 60.84 32.34
			LOT H		
Capalase K Capalase KL Italase Control		56.58 57.50 29.52 27.45	66.24 61.18 38.84 22.49	115.00 90.00 43.49 36. <b>7</b> 1	100.08 123.28 59.68 33.07
			LOT I		
Capalase K Capalase KL Italase Control	36.72 42.70 47.35 34.37	57.61 47.26 44.26 35.62	77.15 71.10 69.32 48.21	90.88 78.24 65.30 43.39	96.35 88.57 66.58 60.40
			MEAN		
Capalase K Capalase KL Italase Control	35.62 37.58 33.17 27.01	50.49 45.88 34.32 27.30	62.71 67.78 48.98 30.28	97.01 84.10 65.57 35.00	86.39 105.91 62.37 41.94
Treated	35.45	43.56	59.82	78.89	84.89
Untreated	27.01	27.30	30.28	35.00	41.94

<sup>\*</sup>  $\mu mol\ FFA/g$  fat in cheese. The data are the averages of duplicate tests.

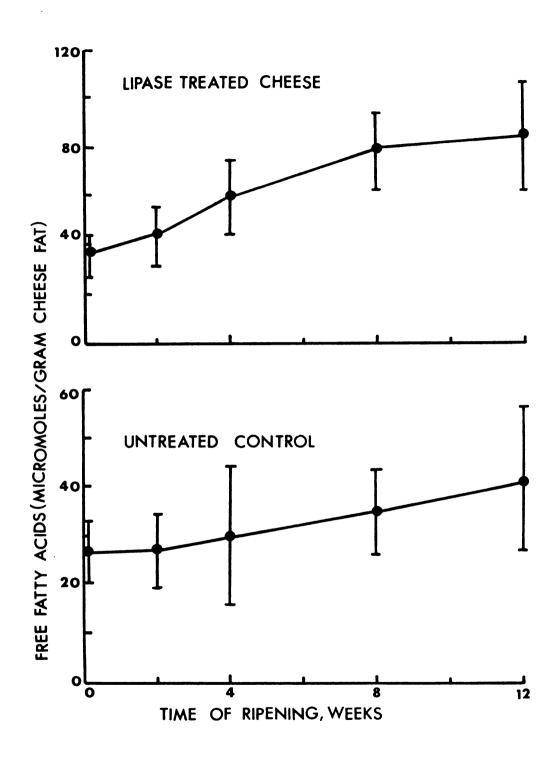


Figure 15. Effect of lipase treatment on free fatty acid content of Latin American White cheese during ripening.

calf. In each case, the lipids are hydrolyzed to yield short chain free fatty acids which impart flavor. The acids also serve as substrates for mold and bacteria in the production of other flavor components (Day, 1967).

In the samples treated with lactic and yogurt cultures, there was a slightly decrease in the FFA titer between 8 and 12 wk of ripening. According to Foster et al. (1957) an initial increase in fatty acids, followed by a decrease, may be due to the utilization of these fatty acids by some organisms in the cheese. Such was the case of the two products where cultures were added.

The cheese treated with lactic culture exhibited slightly higher free fatty acid content as compared to the cheese treated with yogurt culture. This observation may be related to higher lipolytic activity of streptococci as compared to that of lactobacilli reported earlier (Searles et al., 1970).

## Formation of Volatile Fatty Acids

Specially prominent and probably of greatest significance among the hydrolytic products of fat during ripening are the volatile lower fatty acids, including butyric, caproic, caprylic, and capric (Foster et al., 1957). The mode of origin of these compounds is not entirely clear. Lactose fermentation is the source of lactic acid and may give rise, in addition, to the biacetyl, acetic acid and n-butyric acid found in young cheese. Further increase in

acetic acid may result from lactate decomposition or deamination of glycine. However, additional levels of n-butyric, caproic, caprylic and capric acids most likely result from fat hydrolysis in most cheeses.

In this study, the volatile fatty acids (VFA) were separated from the cheese by distillation of an acidified cheese suspension in the presence of MgSO<sub>4</sub>. Neutral alcohol was used to rinse the insoluble acids from the condenser.

The VFA expressed as ml of N/10 acid per 100 g of cheese are shown in Tables 22, 23 and 24, and plotted in Figures 16, 17 and 18. There was an increase in volatile fatty acids in all the treated samples as compared with the untreated control. For cheese treated with lactic culture, mean volatile fatty acid content increased from 7.49 at zero time to 27.13 ml of N/10 acid per 100 g of cheese at 12 wk of ripening. For the cheese treated with yogurt culture the increase was from 6.21 to 34.23 ml of N/10 acid per 100 g cheese after 12 wk and for cheese treated with lipase the volatile fatty acids increased from 17.22 to 30.51 ml of N/10 acid per 100 g cheese.

The results presented in this work show that minor changes also take place in the untreated control during ripening. There was a slight increase in water soluble protein, shear force values, FFA titer and VFA titer. Changes in milk constituents may start at the time of milking in that when the milk reaches the cheese vat a

Table 22. Effect of lactic culture treatment on total volatile fatty acid content of Latin American White cheese during ripening.\*

		ime of Ripenin	
	0	4	12
		LOT A	
Treated Control	6.64 10.98	27.83 13.02	39.07 20.11
001101 01	20.70	13.02	20.11
		LOT B	
Treated Control	8.17 6.13	17.62 10.22	30.38 10.73
	3		20113
		LOT C	
Treated Control	7.66 9.24	29.62 15.09	26.94 12.71
	<b>7.</b>	20000	12.11
		MEAN	
Treated Control	7.49 8.78	25.02 12.78	27.13 14.52
	0.10	12.10	17. <i>76</i>

<sup>\*</sup> All values expressed as ml N/10 acid per 100 g cheese. The data are the average of duplicate tests.

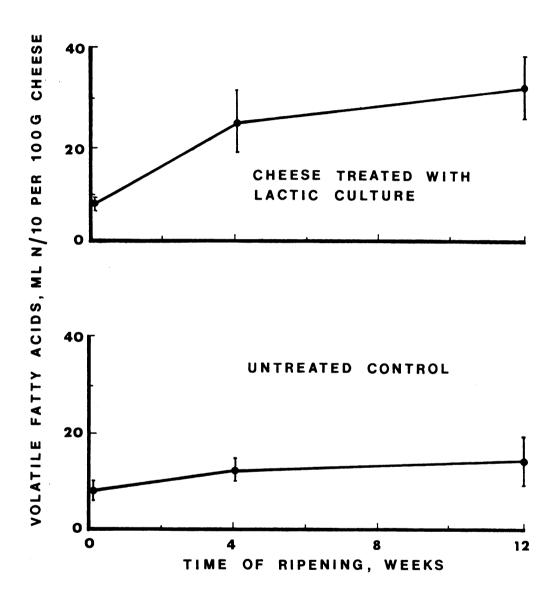


Figure 16. Effect of lactic culture on total volatile fatty acid content of Latin American White cheese during ripening.

Table 23. Effect of yogurt culture treatment on total volatile fatty acid content of Latin American White cheese during ripening.\*

	Time of Ripening (weeks)		
	0	4	12
		LOT D	
Treated	4.98	36.39	36.13
Control	6.12	12.00	11.11
		LOT E	
Treated	8.81	27.19	34.98
Control	4.06	11.24	16.85
		LOT F	
			_
Treated Control	4.85 9.70	17.75 10.30	31.56 11.54
		MEAN	
Treated	6.21	27.11	34.23
Control	6.63	11.18	13.43

 $<sup>^{*}</sup>$  All values expressed as ml N/10 acid per 100 g cheese. The data are the average of duplicate tests.

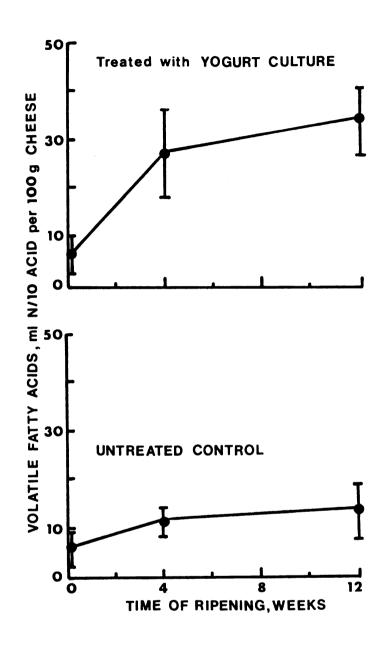


Figure 17. Effect of yogurt culture treatment on total volatile fatty acid content of Latin American White cheese during ripening.

Table 24. Effect of lipase treatment on total volatile fatty acid content of Latin American White cheese during ripening.\*

	<del> </del>		
	0	Time of Ripening 4	(weeks)
		7	± C
		LOT G	
Capalase K Capalse KL Italase Control	15.20 18.89 17.93 5.52	27.32 34.98 16.85 9.45	31.41 22.73 27.83 14.04
		LOT H	
Capalase K Capalase KL Italase Control	17.11 18.20 17.61 4.60	27.28 29.10 26.92 9.59	32.22 36.42 28.10 14.19
		LOT I	
Capalase K Capalase KL Italase Control	16.92 17.23 16.00 4.89	26.99 33.12 22.31 10.13	32.89 34.16 28.84 16.20
		MEAN	
Treated Untreated	17.22 5.00	27.21 9.72	30.51 14.81

<sup>\*</sup> All values expressed as ml N/10 acid per 100 g cheese. The data are the average of duplicate tests.

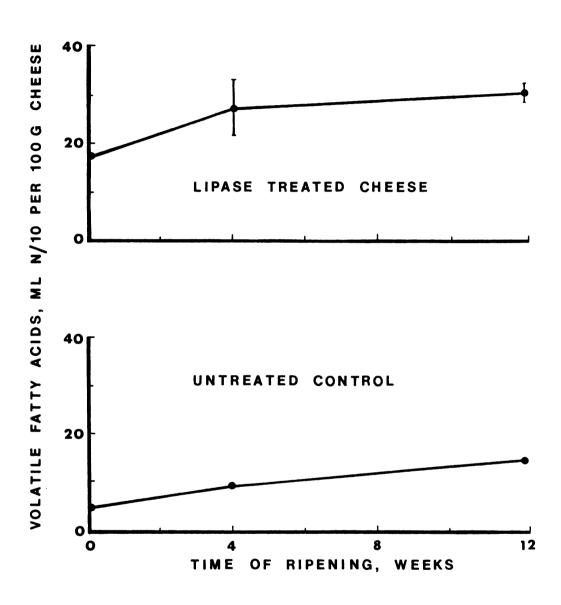


Figure 18. Effect of lipase treatment on total volatile fatty acid content of Latin American White cheese during ripening.

bacterial flora is already established in the milk. All those organisms or their enzymes capable of surviving the heat treatment may be involved in the changes observed during ripening.

## Organoleptic Evaluation of Latin American White Cheese

The development of new flavor and texture characteristics in Latin American White cheese by addition of lactic culture, yogurt culture and lipase enzymes to the cooled curd was assessed by organoleptic evaluation of the products at 2, 4 and 6 wk of ripening.

First, a panel of 4 trained judges, scored the cheese samples for flavor, body and texture according to the ADSA form for evaluation of Cheddar cheese.

The flavor scores presented in Table 25 show a slight preference for 4 wk aged cheese as compared to cheese ripened for 2 and 6 wk.

Cheese ripened for 4 wk did not have any serious flavor criticism. The control was considered flat but it had a high score of 8.25 out of 10 points. In general, yogurt cultured cheese received the highest flavor score, while the one treated with lactic culture received the lowest score. The cheese treated with lactic culture was considered acid and unclean in flavor. Cheese treated with lipase enzymes received variable flavor scores depending on the type of lipase used in the treatment. It was con-

Table 25. Comparison of flavor scores in samples of Latin American White cheese at 2, 4 and 6 weeks of ripening.

Sample	Average Flavor Score	Remarks	
	2 WEEKS	S	
Yogurt culture Lactic culture Capalase K Capalase KL Italase Control		Flat, acid Flat, acid Fermented, sulfide, acid Flat, unclean Fermented/fruity, whey taint Flat, heated	
4 WEEKS			
Yogurt culture Lactic culture Capalase K		Flat, heated Acid, unclean Whey taint, fermented/fruity, foreign	
Capalase KL Italase Control	8.00 8.00 8.25	Flat, whey taint, slightly rancid Flat, heated, fermented/fruity Flat	
	6 WEEKS	S	
Yogurt culture	8.00	Little rancid, flat, whey taint, stale NFDM	
Lactic culture	7.25	Very acid, whey taint, stale NFDM, fermented/fruity	
Capalase K	.7.00	Little rancid, flat, whey taint, fermented/fruity	
Capalase KL	7.50	Little rancid, acid, whey taint, fermented/fruity	
Italase	7.00	Whey taint, fermented/fruity, acid, rancid	
Control	8.50	Flat, acid, heated	

<sup>\*</sup> The flavor scores represent the average of 4 trained judges. Perfect score = 10 points.

sidered fermented/fruity and slightly rancid. Compounds responsible for the production of fruity flavor in Cheddar cheese have been identified by Bills et al. (1965). This defect was attributed to the excessive alcohol production and the subsequent formation of ethyl esters of FFA. The fruity/fermented and rancid characteristics observed in cheese treated with lipase enzymes after 4 wk appear in all the treated cheese after 6 wk of ripening. The fact that these characteristics appear earlier in this cheese may be due to relatively higher lipolysis observed earlier.

Cheeses aged for 6 wk were scored lower in flavor than other samples. They were considered rancid, fermented/ fruity with whey taint. This last term describes offflavor in Cheddar cheese associated with whey (Nelson and Trout, 1964). Whey flavor would be a normal component of a cheese like Latin American White cheese. Often cheese containing whey has body and texture characteristics of a high moisture cheese. The control cheese was considered flat and heated. Since the white cheese is made at elevated temperatures (82 C), it is likely to exhibit cooked flavor. The control cheese had the highest flavor score after 6 wk or ripening, while the cheese treated with lactic culture or lipase enzymes had a lower score. It seems that 6 wk of ripening is excessive for all treated samples of the white cheese. Among the treated cheeses ripened for 6 wk, yogurt treatment resulted in a flavor score better than other cheeses.

In the case of cheeses ripened for 2 wk, the flavor was, in general, considered flat. Only cheese treated with Italase enzyme preparation was considered fermented/fruity. Scores for cheeses ripened 2 wk were higher than the ones for cheese ripened for 6 wk. Cheese treated with lactic culture had lowest flavor score. Evan at 2 wk of ripening it was considered acid.

For body and texture evaluation, a similar trial was conducted. Results are shown in Table 26. The average score for body and texture was relatively low for all cheeses at 2, 4 and 6 wk of ripening. The highest body and texture score was for cheese treated with yogurt culture, after 4 wk of curing.

All cheeses, including control, were considered mealy, and corky and in most cases open and short. The texture in cheese treated with lactic culture was especially identified as very crumbly. This may be due to the high acidity of the product (pH 5.21 to 4.94). A mealy cheese is the one from which the fat seems to be released easily. It generally has a short body with little elasticity. A corky cheese is generally a low-fat or young cheese. These defects are related to Cheddar cheese. Latin American White cheese is a relatively different product. The white cheese contains lower fat, is relatively unripened and possesses little elasticity due to the absence of rennet. As a consequence, it should be considered to

Table 26. Comparison of body and texture scores in samples of Latin American White cheese at 2, 4 and 6 weeks of ripening.

Sample ar	Average Body nd Texture Sco	ore* Remarks
	2 WEE	KS
Yogurt culture Lactic culture Capalase K Capalase KL Italase Control	3.00 3.75	Corky, gassy, crumbly, mealy Very crumbly, open, short, mealy Corky, mealy, crumbly, open Mealy, corky, curdy Mealy, corky, curdy Mealy, corky, gassy, crumbly
	4 WEER	KS
Yogurt culture Lactic culture Capalase K Capalase KL Italase Control		Mealy, corky, crumbly Very crumbly, corky, mealy Curdy, corky, gassy, crumbly Corky, curdy, mealy, open, short Corky, crumbly, mealy Mealy, open, short
	6 WEER	KS
Yogurt culture	3.25	Open, crumbly, curdy, mealy, short
Lactic culture	3.25	Very crumbly, very open, mealy,
Capalase K	3.00	curdy, very short Mealy, crumbly, weak, curdy,
Capalase KL	3.00	open, short Mealy, crumbly, weak, curdy,
Italase Control	3.50 4.00	open, short Mealy, corky, curdy, open, short Mealy, corky, curdy, open, short

<sup>\*</sup> The body and texture scores represent the averages of 4 trained judges. Perfect score = 5 points.

possess different but normal body and texture characteristics.

In the second phase, 123 non-trained tasters were asked in a triangle test to differentiate 3 samples (two alike, one different) of cheeses ripened for 2, 4 and 6 wk. The results are presented in Table 27.

After 2 wk of ripening period, the taste panel was unable to differentiate cheese treated with yogurt culture, Capalase KL or Italase from an untreated control. For the cheese treated with lactic culture, the panel was able to differentiate it from the untreated control at a 0.1% probability level of significance. For the cheese sample treated with Capalase K enzyme preparation, the tasters were able to differentiate from untreated control to a 5% level of significance.

After 4 wk ripening, the panel was still unable to differentiate the cheese treated with yogurt culture and Italase from the untreated control. Cheese treated with Capalase K enzyme preparation which was differentiated from the control at 2 wk of ripening, appeared to have no significant difference from the control after 4 wk.

Cheese treated with lactic culture was differentiated from the untreated control by all the tasters. In the case of cheese treated with Capalase KL, the panel was able to differentiate from the control at a 0.1% level of significance.

After 6 wk of ripening all treated cheeses except that

Table 27. Sensory evaluations and differences in treated and untreated Latin American White cheeses.

Treatment	Observed Difference from Untreated Control	Probability Level of Significance
Yogurt culture Lactic culture Capalase K Capalase KL Italase	2 WEEKS  NSl  Significant Significant NS NS	0.1 5.0
Yogurt culture Lactic culture Capalase K Capalase KL Italase	4 WEEKS  NS Significant NS Significant NS	0.1
Yogurt culture Lactic culture Capalase K Capalase KL Italase	6 WEEKS Significant Significant Significant Significant NS	1.0 0.1 0.1 1.0

<sup>1</sup> Not significant.

treated with Italase enzyme preparation were significantly different from the untreated controls.

In the preference tests, after 2 wk of ripening, no preference between treated and untreated cheeses was observed. Only in the case of the cheese treated with lactic culture, there was a significant perference for the control. After 4 wk of ripening, yogurt cultured cheese was markedly preferred over the control, while in the case of cheeses treated with lipase enzymes the control was preferred. Lactic cultured cheese and control did not have differences in preference at 4 wk of ripening. Among cheese samples ripened for 6 wk, the control was always preferred. However, more cheese flavor was detected in treated cheeses at 4 and 6 wk of ripening period. In general, the control was reported smoother by the panel.

Some comments were:

<sup>&</sup>quot;differences are small"

<sup>&</sup>quot;odd sample (lactic culture, 6 wk) is awful, highly acidic taste"

<sup>&</sup>quot;the paired sample (yogurt culture, 6 wk) had a bit of bitter aftertaste"

<sup>&</sup>quot;odd (lactic culture) is more fermented and sour"

<sup>&</sup>quot;excellent (lipase K treated, 6 wk)"

<sup>&</sup>quot;odd sample (K, 6 wk) does not have very good flavor"

<sup>&</sup>quot;odd sample (K, 6 wk) has more intense flavor but a bit unpleasant"

<sup>&</sup>quot;texture of odd sample (control) preferred"

<sup>&</sup>quot;odor quality difference"

- "I like the taste and flavor of these samples. This reminds me of the cheese that I buy in my country (Iran)."
- "the paired samples (lactic culture, 6 wk) are quite easy to bite"
- "the odd sample (control) tastes a little stale"
- "the matched samples (lactic culture, 6 wk) are too acidic to be edible"
- "none of them is particularly good"
- "can't tell the difference (Capalase K, 2 wk)"
- "odd sample (Italase, 2 wk) slightly fruity"
- "paired samples (Italase, 4 wk) seemed more flavorful"

## SUMMARY AND CONCLUSIONS

Eighteen 195-kg lots of whole milk were heated to 82 C and the protein precipitated by adding 2.39 g citric acid per kg of milk. To modify flavor and texture, lactic culture, yogurt culture and lipase enzymes were applied to the curd prior to pressing. Vacuum packed blocks were ripened at 10 C. Physico-chemical changes in control and treated cheeses were measured over a 12 wk period. The following were the findings:

- 1. In most cases the number of microorganisms reached a maximum in the first two weeks of ripening. Then a decrease was observed between 4 and 8 wk with a slight increase at 12 wk of curing.
- 2. The protein breakdown in Latin American White cheese as function of time was slight but progressive. For cheese treated with lactic culture water-soluble protein increased from 0.83 g to 1.11 g per 100 g cheese during 12 wk of ripening. Cheese treated with lipase enzymes did not have appreciable increase in soluble protein. Highest proteolysis was observed when yogurt culture was used. In this case, 0.94% of protein was water soluble at zero time and the value increased to 1.18% after 8 wk of ripening.
  - 3. The shear force values show a slight increase in

the firmness in all, treated and untreated, cheese with increase in time of ripening. The increase of firmness as shear-force value was 22 and 14% for cheese treated with lactic culture and lipase enzymes, respectively, after 12 wk of ripening. The white cheese treated with yogurt culture showed the least increase in firmness (7.35%) in 12 wk.

- 4. The pH of Latin American White cheese decreased with increase in the time of ripening. Although rate of decrease differed somewhat for the individual cheeses, the pH values were within pH 4.9 to 5.8. The pH of cheese treated with lactic culture was generally lower (5.21 to 4.94).
- 5. Lipolysis in Latin American White cheese was measured by determination of free fatty acid titer and formation of volatile fatty acids. In both cases an increase in free fatty acids was observed with the time of ripening. There was a marked difference in the increase of free fatty acids in the cheese treated with lipase enzymes (58% increase) as well as in cheese treated with lactic and yogurt culture.
- 6. The flavor, body and texture of the cheese treated with yogurt culture, after 4 wk of ripening received the highest score. All cheeses aged for 6 wk were scored lower than other samples ripened for 2 and 4 wk. They were considered rancid, fermented, fruity and with whey taint. Cheeses ripened for 2 wk were considered flat in

flavor. The control was always considered flat. Cheese treated with lactic culture was always considered highly acidic and crumbly in body. All cheeses, including control, were considered mealy, corky, open and short. Only after 6 wk of ripening was the taste panel able to differentiate most of the treated samples from the untreated control. Cheeses treated with yogurt culture and Italase did not have significant differences after 4 wk of ripening. Only cheese treated with lactic culture was differentiated after 2, 4 and 6 wk of age from the untreated control.

In conclusion, Latin American White cheese treated with lactic culture, yogurt culture and lipase enzymes undergoes appreciable changes with ripening time. The changes may improve organoleptic characteristics if the amount added and the time of ripening are controlled.

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