CHANGES DURING RIPENING OF UNSALTED CHEDDAR CHEESE

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ABSTRACT

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By

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Absence of salt in Cheddar cheese can result in a different pattern of ripening. The prime purpose of this research was to explore the extent of some salient chemical and physical changes occurring in unsalted Cheddar cheese over a ripening period of 12 wk. Results were compared with those in the salted controls made from the same batch of cheese curd.

The flavor of unsalted Cheddar cheese was described as flat, fermented, bitter and unnatural. The body and texture was judged to be weak, pasty and gassy. At the end of the 12 wk ripening period, the unsalted cheese had an average flavor score of 36.37-37.20 compared to 39.20-39.50, for the controls, and the body score of 26.87-27.30 compared to 29.00-29.37, for the controls. With the incorporation of 0.5-1.5% salt into the ripened unsalted cheese, the flavor score increased and with 1.0% salt, compared closely to the score of the controls.

A precise evaluation of the change in the firmness of the unsalted cheese during ripening was made with a Lee-Kramer Shear Press. The initial shear force value for the unsalted cheese was 0.1302 lb/g (maximum). At the end of 10 wk the unsalted cheese registered a value of 0.0890 lb/g (minimum). The shear force values for the salted controls showed an initial value of 0.2433 lb/g, a maximum of 0.3082 at 1 wk and 0.2503 (minimum) at

10 wk. After decrease for 10 wk there was no significant change observed in the average shear force values.

The average pH value for the unsalted cheese increased from 5.16 at 1 wk to a maximum of 5.29 at 7 wk and then variations were observed to 12 wk. During ripening for 12 wk the samples of unsalted cheese showed a lower pH than the corresponding controls. Progress in the ripening of the cheese also was measured by determining nitrogen in water-soluble nitrogen compounds, which rose from an initial value of 6.00-8.00% in the unsalted cheese to 36.00-41.10% after 12 wk. The corresponding values for the salted controls varied from 4.60-7.15% to 21.40-29.50% during the same period.

The average volatile fatty acids in the unsalted cheese steadily increased from 22.90 ml to a maximum of 28.87 ml of N/10 acid per 100 g cheese at 12 wk. Increase in the volatile fatty acids of salted cheese was from 16.70 ml to 22.63 ml of N/10 acid per 100 g cheese at 8 wk. During the subsequent ripening although some variations in the amount of volatile fatty acids in the unsalted and salted cheese samples were observed, the unsalted cheese showed higher values than the corresponding controls at each interval of testing.

The acetic acid content of unsalted cheese increased from an average value of 427.8 mg/kg cheese to 515.5 mg/kg at 10 wk. Corresponding values for the salted cheese were 285.7 mg/kg and 386.1 mg/kg at 8 wk. At 14 wk ripening, the acetic acid content for unsalted cheese had increased over 10 wk and was much higher than content for the salted cheese.

Gas chromatographic analyses of free fatty acids in unsalted and salted cheese samples showed that identical, even-numbered carbon chain fatty acids were liberated during ripening. The quantitation of the individual fatty acids revealed that myristic, palmitic, stearic and oleic acids were

liberated in higher quantities than the rest of the fatty acids in the samples of cheese analyzed during the 12 wk ripening. Also, the overall hydrolysis of fat in the unsalted cheese was found to be more extensive than in the salted controls.

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DEDICATION

This dissertation is dedicated to the fond memory of my late elder brother, Mr. Rup Kant Thakur, M.A., Dip-in-Ed., whose energy, courage and inspirations will always be symbolic.

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CHAPTER I

INTRODUCTION

Doctors have been recommending a sodium restricted diet for patients suffering from kidney trouble, hypertension, congestive heart troubles, cirrhosis of liver with fluid retention, or toxemia of pregnancy. Since 1929 salt intake has been believed to be associated with blood pressure. In recent years the theory that excessive sodium intake over an extended period of time might result in hypertension and atherosclerosis in susceptible individuals, is accepted by the medical profession. The harmful effects of excessive use of salt apparently was known for several centuries. A documented reference dating back to 1572 indicates that Thomas Tusser, a poet and a specialist in the farming problems mentions, "..much saltness in white meat is ill for the stone." A highly salted 'white-meat' (cheese) was found to harm the patients with 'stone' (a kind of kidney disease).

To many people cheese is an important source of protein and calcium. However, dietary limits of 500-1000 mg of sodium per day is commonly recommended for many people. A 100 g slice of Cheddar cheese contributes roughly 600-1500 mg sodium. Sodium restrictions also limit the intake of meats, fish and poultry. Unsalted cheese because of its low sodium content (approx. 25-55 mg/100 g cheese), high nutritive value and a wide variety of uses in foods has potential for many applications in sodium restricted diets.

Experimental evidence with rats showed that the intake of calcium

initiated an interaction between calcium and sodium in a manner that if more calcium was excreted more sodium was excreted (Saunders, 1970). Thus, unsalted cheese should be a valuable adjunct to sodium restricted diets.

No comprehensive investigation has been reported on the flavor attributes and other physico-chemical ripening aspects of salt-free Cheddar cheese. This study, therefore, was undertaken to investigate some of the salient changes in this type of cheese during a ripening period of up to 12 wk. The primary objective of this study was to determine the effect of the absence of salt in cheese on:

1. production of free fatty acids;
2. degradation of proteins;
3. overall organoleptic evaluation of flavor and body; and
4. body firmness by measuring the peak pound force resistance to shear the cheese sample, by employing a Lee-Kramer Shear Press.

CHAPTER II

REVIEW OF LITERATURE

Status of Low Sodium Foods

Salt has been used in cooking and preservation of foods since prehistoric time. The appetite for salt and other additives containing sodium seems to be an acquired habit rather than a reflection of need, although sodium and chloride are necessary for man as constituents of extracellular fluids in the body (Gormican, 1972). Functions of sodium and chloride ions have been discussed in greater detail by Davidson (1954):

1. Sodium in the extra-cellular fluids participate in maintaining osmotic equilibrium (water balance) between the solutes of extra-cellular fluids and solutes contained within the cells (fluctuations from osmotic equilibrium may have dire results upon the functional capacity and the viability of the cells);

2. through its function as a buffer base in conjunction with bicarbonate and phosphate, sodium maintains pH of blood within normal limits;

3. sodium alone or in conjunction with other extra-cellular ions functions to control nervous impulses, muscle contractility and especially the conduction and contractility of heart muscles.

Salt utilization varies considerably among individuals and various countries of the world. Saunders (1970) correlated the high salt intake of several populations around the world with the incidence of arterial hypertension. Achard and Leoper (1901) demonstrated that quantity of salt

ingested was related to the edema formation in patients with heart disease. Then in 1929 they further postulated that low salt diet seemed to be found fairly frequently with normal blood pressure and the converse was likewise true.

While a healthy kidney has the capacity to excrete sodium, there also appears to be growing support for the thesis that excessive sodium intake over a period of time might cause hypertension and atherosclerosis in susceptible individuals (Dahl, 1961). There are a number of clinical conditions in which sodium retention occurs, e.g. congestive heart problems, hypertension, kidney disease, cirrhosis of liver with fluid retention, severe malnutrition and toxemia of pregnancy (Gormican, 1972). Strict adherence to a sodium restricted diet may become a life or death proposition.

As early as 1953, a method was developed by Chaney in cooperation with Los Angeles County Heart Association, which, according to Heap (1968), removed up to 90% of the original sodium content of fresh milk (approximately 120 mg sodium/8 oz milk). Since sodium restriction essentially limits the intake of meats and other high-protein foods that normally have high sodium content, the enthusiasm for sodium restriction has to be paralleled with an equal zeal for providing the necessary nutrients. White (1957) stressed that the wealth of nutrients furnished by milk especially protein, calcium and riboflavin was no less important in sodium restricted diets. He also suggested that other protective foods with high sodium content could be replaced with low sodium milk.

Unsalted Cheese

Cheese is an important part of the diet of many Americans. This food has a high caloric value, is easily digested and is rich in protein and fat.

However, implication of high sodium intake in certain disease conditions make the use of salted cheese objectionable. Efforts, therefore, were made to produce unsalted cheese despite the common knowledge that salt renders it more flavorful and is necessary for normal ripening.

High calcium content of the unsalted cheese may have an added advantage if included in the diet of the patients who need to get rid of excess sodium. This conclusion may be based on the experimental data of Saunders (1970) who found that a greater secretion of calcium was accompanied by a greater secretion of sodium in experimental rats which were fed different doses of calcium and normal saline. Sharara (1956) also reported an increasing loss of calcium in the cheese whey as the percentage of sodium chloride in the curd was increased to a certain limit. The effect was discussed in relation to the exchange of calcium in the casein for sodium.

In want of an improvement in variety for a palatable, balanced diet with restricted sodium content, unsalted cheese may possibly be used.

Details of any comprehensive analysis on physico-chemical aspects of unsalted cheese apparently has not been reported in literature except for the processed "dietary" cheese produced in Holland, which was analyzed and found to contain as high a sodium content as natural salted cheese; possibly due to the use of 'emulsifiers' containing sodium (Mulder and Schouten, 1955).

Effect of Salt on Some Important Physico-Chemical Properties of Cheese

Salt in Cheese

Salting of cheese has been considered an essential step for almost all ripened varieties. Tustin, Jr. (1946) stated that the aim of salting

was to get a good quality cheese with maximum yield and minimum shrinkage during ripening. Davies, et al. (1937) showed that among a number of externally added chemical substances likely to affect the growth and metabolism of bacteria or the rennet activity, the largest effect on the rate of chemical ripening was exerted by the rennet and salt. Marquardt and Yale (1941) found the salt content of most varieties was between 0.7-2.0%, but the concentration of salt in the aqueous solution was much higher.

Salt concentration affects bacterial growth and thus the ripening of cheese (Tittsler, 1965). Irrespective of the method of salting, the salt concentration in the surface layer may reach 16% or greater. This level of salt is approximately the limit that can be endured by microorganisms with the exception of a few halophilic types (Davis, 1965). Functions of salt in cheese are to (1) suppress growth of undesirable microorganisms, (2) assist the physico-chemical changes in cheese, (3) retard the growth of lactic acid and other desirable types of microorganisms, (4) influence the firmness of cheese and (5) make cheese more tasteful (Marth, 1963).

Physical Changes Influenced by Salting.

Moisture Retention of Curd.

Salt has a marked effect on the moisture content of the curd. In the past, correcting an overmoist curd was accomplished by the addition of salt (Tustin, Jr., 1946). Cheesemakers know that an immediate release of whey from the curd follows the addition of salt. The effect of salt is to cause contraction of the curd and release of moisture. High levels of salt result in lower moisture values, stickiness and retarded

ripening (Reynolds, 1946). The omission of salt results in high moisture cheese which promotes rapid ripening (Tittsler, 1965, Davis, 1965).

Moisture content of the curd at definite stages during cheese making along with the acidity are important factors controlling the quality of the product. Differences in these factors are responsible for the different texture and body values and these differences are influenced by moisture content of the cheese.

Body and Texture of Cheese.

Salting produces an immediate increase in the hardness of the curd (Csiszar, 1949) and salted curd mats more readily. Undersalting and oversalting adversely affect the moisture of cheese. A low salt content results in higher moisture and more likely a pasty and/or weak body and open texture (Riddet, et al., 1933 and Irvine, 1955). Studies conducted to assess the effect of salt on the development of 'Dutch' type cheese showed that the characteristic, elastic and plastic properties of the cheese were influenced by the water distribution and by the extent of proteolysis of the paracasein complex; both of which are regulated by the presence of salt (Ramanauskas, 1971). Oversalting causes dry, brittle body, cracking of cheese rind which may result in mold growth within the cheese (Tustin, Jr., 1946). The presence of salt has also been shown to influence the solubility of nitrogenous compounds in the cheese because salt decreased the hydrolysis of paracasein due to the decrease in the number of the lactic acid bacteria (Sandberg, et al., 1930).

Failure to salt the curd evenly results in cheese with portions having low salt, which tend to become the regions of off-flavor including putrefaction. The rind may also become soft and the cheese misshapened (Davis, et al., 1937).

Chemical Changes Influenced by Salting.

During ripening of cheese, protein, fat and to a less extent lactose undergo complex biochemical reactions, which are responsible for the development of typical body, texture and flavor of the cheese. The changes during ripening involve fermentation of lactose, partial hydrolysis of proteins, peptides and fat. The mechanism responsible for these different biochemical reactions are catalyzed by enzymes from the microorganisms produced during the ripening process and to a lesser extent enzymes which are specific in the 'cheese substances' (Schormuller, 1968). The source of the latter are starter microorganisms added in the cheese making, milk and the rennet (Tittsler, 1965). Important influences on these enzymes are the dynamic changes in the composition and pH of the ripening cheese, as well as the decomposition and the end products of these reactions (Schormuller, 1968).

pH

Changes in pH are important to the ripening process. Lactic acid produced in cheese has a physico-chemical action during ripening. It regulates the pH level and ion equilibrium (Schormuller, 1968). The gradual increase in pH during storage is caused by destruction of lactic acid to nonacidic decomposition products and less highly dissociated acids, which include acetic and carbonic acids, as well as, alkaline products of protein decomposition.

pH changes primarily affect the characteristics of the curd as the body is controlled by 'acid-base equilibrium' and enzyme activity (Davis, 1950). Every enzyme has an optimum pH for activity. Therefore, enzyme reactions fluctuate when the pH of the media deviates from its optimum point (Gale, 1951).

Sodium chloride is known to affect the activity of the enzyme system or the pH value, which directs the ripening process in a particular direction (Schormuller, 1968). Since the omission of salt results in a cheese of higher moisture content, more lactose is retained in the curd which may be available for the production of lactic acid. In an effort to correlate composition, pH and the color of the Cheddar cheese, Irvine (1951) found that 'acid' cheese had a lower pH, high moisture and low fat content. Raadsveld and Mulder (1949) indicated that the hydrolysis of fat was found to take place more quickly in cheese with low pH than in cheese with high pH. In another set of experiments on the reaction of glutamic acid and glycine in the ripening skim milk cheese, using the same species of microorganisms, Schormuller and Leichter (1955, a, b) found that the breakdown of amino acid is dependent not only on the species of microorganisms but also on the pH of cheese.

Lactose Fermentation.

Lactose in cheese although present in small quantities, may be important in that it serves as a primary energy source of the lactic bacteria. The changes observed occur first to lactose, then to the decomposition products of the disaccharide, galactose and glucose and finally to calcium lactate (Schormuller, 1968). Alfredsson, et al., (1962) found that damaging the homofermentative starter bacteria with high scalding temperature was reflected by higher galactose content and less formation of lactic acid.

Lactic acid development repressed the growth of harmful and undesirable organisms. The amount and rate of production of this acid influenced the quality of cheese. Its role was different in the development of various varieties. Harper and Kristoffersen (1956) reported that

the glycolysis of lactose to lactic acid occurs with many intermediate stages.

Effect of salt on the fermentation of lactose in cheese does not seem to have been investigated. However, the absence of salt obviously would influence the lactose fermentation by the microbial flora.

Proteolysis.

Proteins in cheese are known to undergo degradation to varying extents depending upon the variety of cheese and degree of curing. Proteins provide much of the physical structure, body and textural properties. Casein fractions, the main protein in cheese, are degraded by a mechanism that is common to most varieties. Amino acids are considered to contribute to the flavor of cheese, but their contribution to the characteristic flavor is not certain (Harper and Kristoffersen, 1956). Qualitatively, the amino acids released by proteolysis during the ripening of cheese are the same as those present in the casein (Kosikowski, 1951) and (Kosikowski and Dahlberg, 1954). These amino acids contribute to the substrate for the cheese flora and may produce flavorful compounds. The amino acids of the cheese protein are also of special importance in the formation of a number of fatty acids (Schormuller, 1968).

The rate, nature and extent of protein decomposition during cheese ripening are influenced by the kind and concentration of microbial enzymes, moisture content (Sammis and Germain, 1929), presence of lactic acid (Zaykowski and Slobodska-Zaykowsky, 1925), temperature (Sanders, et al., 1946 and Van Slyke and Hart, 1903), pH, oxidation-reduction potential and salts that affect enzyme activity. The reaction is basically the same whether the hydrolysis involves an acid, a base or an enzyme:

$$R\ CO\ NH\ R' \xrightarrow{+H_2O} R\ COOH + R'NH_2$$

Higher moisture and low salt concentrations increase the rate and extent of proteolysis (Van Slyke and Hart, 1903). Sodium chloride when added in increasing amounts not only decreased the number of streptococci in cheese, but also diminished the amount of 'soluble nitrogen'. Davis (1965) reported that salt affects the mechanism of cheese ripening in the following manner:

1. activates the proteolytic enzymes of rennet,
2. brings the protein fraction into solution for enzyme degradation,
3. changes the acid-base equilibrium in cheese, and
4. alters the proportion of bound and free water.

Acetic Acid in Cheddar Cheese.

Despite differences in opinion among researchers as to the substrate for acetic acid production, it is generally agreed that this acid is the product of bacterial metabolism. Suzuki, et al., (1910) reported acetic acid was formed from the lactates present in cheese and reached a maximum at 3 mo. after which a decrease was noted. Honer (1953) noted a definite cyclic change in the concentration of acetate in cheese during ripening. It increased, then decreased, again increased and then decreased again. Fruton and Simmonds (1960) have suggested that species of lactobacilli, and pseudomonas and E. coli form acetic acid by beta-oxidation of fatty acids. Kandler (1961) proposed a mechanism by which starter bacteria derived this acid directly from the utilization of carbohydrates. Ohren (1965) stated that acetic acid in Cheddar cheese could be derived from carbohydrate, amino acids or fatty acids. When mixed lactic starter cultures used for Cheddar cheese were grown in milk, lactic acid and small

amounts of acetic and propionic acids were formed. Nakae and Elliott (1965, a, b) demonstrated that S. lactis C_2 formed acetic acid from alanine and serine by deamination and decarboxylation. They also reported that microorganisms will deaminate and decarboxylate other amino acids to form various volatile acids.

Fat Hydrolysis.

The fact that milk fat is essential for the development of typical flavor of cured cheese is accepted by researchers. The nature of the decomposition of fat in cheese largely depends on the variety and is more extensive in mold inoculated curd. The numerous volatile and nonvolatile carbonyl compounds present in cheese indicate that fatty acid oxidation might occur in addition to the lipolytic changes in fat due to lipases.

Suzuki, et al. (1910) isolated a distillate, possessing the flavor of aged cheese, which contained alcohols, esters and fatty acids. The non-nitrogenous products were considered to have been derived from cheese fat during ripening. Lane and Hammer (1939) showed that, as cheese ripened, the acidity of cheese fat increased and was higher in raw milk cheese than in pasteurized milk cheese.

Mattick and Hiscox (1939) related levels of acids in cheese to its microflora and concluded that a high volatile acid content was associated with the presence of high numbers of non-lactic bacteria.

Bills and Day (1964) observed that fat hydrolysis was a normal chemical change taking place in cheese. Dacre (1955) found that ethyl acetate and ethyl butyrate, both esters of fatty acids, were present in Cheddar cheese. Day (1967) also reported that lactones, methyl ketones, esters, alcohols and fatty acids were derived from milk fat during cheese ripening.

Hydrolysis in milk fat occurs in cheese due to action of lipases which may originate from milk and the microorganisms. While lipase originally present in milk has little effect on fat hydrolysis in cheese, a significant influence of microbial lipase on fat hydrolysis was found (Stadhouders and Mulder, 1953). Gould (1941) reported that sodium chloride had an inhibitory effect on milk lipase in raw homogenized milk and cream, and amounts of 5-8% completely inhibited lipolysis. Factors affecting lipolysis were: salt, pH and storage conditions (Stadhouders, 1956). Later, Chandan and Shahani (1964) also noted that various salts, for example, sodium chloride, zinc chloride, magnesium sulfate and magnesium chloride were inhibitory to milk lipase. Stadhouders and Mulder (1957) further reported that microorganisms representative of the Alkaligenes, Achromobacter, Pseudomonas and Serratia groups if added to pasteurized milk, increased the hydrolysis of fat in cheese made from this milk.

Jensen (1964) found that in the hydrolysis of mixed triglycerides enzymes exhibited a distinct preference for the fats containing short chain fatty acids. Milk lipase has a preference for the release of fatty acids from the primary positions of a triglyceride. Butyrate being a primary ester, often was preferentially released. Lauric and higher acids were the major acids released at all stages of lipolysis in an experiment of Harwalkar and Calbert, (1961) but with the advancement of lipolysis the ratio of these acids to butyric decreased.

Microbiological Aspects.

Bacterial counts in cheese vary widely but inversely with the salt content (Hoecker and Hammer, 1944). Sodium ions are relatively more toxic than the chloride ions but a concentration of 5% salt in cheese was not

sufficient to inhibit growth of certain bacteria. E. coli required 12% salt for inhibition, while 3% had a stimulating effect (Davis, 1965). Earlier Hof (1935) found that only a few species could tolerate 6% salt and very few could survive 15%. Starter streptococci were not affected by up to 2% salt but were almost completely inhibited by 5% (Platon, 1942). Sjostrom (1944) observed that some starters may be affected even at 2% salt concentration. Walter, et al., (1958) reported that S. lactis was generally not inhibited by less than 1.6% and not significantly at 1.6-2.0% salt. Walter, et al., (1958) also found that S. cremoris was inhibited slightly by 1.6% and almost completely by a 2.0% salt, while a mixture of S. lactis and S. cremoris seemed to resist sodium chloride more uniformly than single strains. Malushko (1957) noticed that 0.5-2.5% salt had an inhibitory effect on gas producing bacteria in 'Dutch' type cheese made from raw milk; lactic acid bacteria appeared to have been affected less.

Davis (1965) noted that a higher concentration of salt at the surface than in the interior of the curd exerted an inhibitory effect for several hours. He also indicated that apart from the inherent salty taste of the cheese, quite different flavors would be obtained if cheese was made without salt, because of the different microflora that developed. The production of the common proteolytic and lipolytic bacteria would be far higher and the cheese would almost certainly possess an unpleasant odor and taste. These bacteria normally constitute about 1% of total microflora in good salted Cheddar cheese.

Davis and Mattick (1932) suggested that the state of nitrogen in cheese may be a controlling factor. They found that digestion of protein beyond a certain point resulted in an inhibition of lactic streptococci.

Studying the effect of salt concentration of the microflora of cheese from the public health view point, Wagenaar and Dack (1953) noted that the salt requirement necessary to inhibit toxin production by C. botulinum decreased with the length of ripening. In order to study the survival of some pathogens in Cheddar cheese, Campbell and Gibbard (1944) experimented with S. typhosa and Yale and Marquardt (1940) with S. pyogenes. They found that an increase in ripening temperature reduced the survival period of pathogens in both cases. S. typhosa was not affected either by the amount of starter or the acidity of cheese. Mattick, et al. (1959) could not recover S. aureus, added to cheese milk, from any of the Cheddar cheese samples after 14-22 wk. They further related the results with the acidity of cheese during manufacture and reported that S. aureus died out least rapidly in the cheese in which the least amount of acidity was allowed to develop during manufacture.

Salt, acidity, temperature and oxygen are factors influencing the growth of microorganisms. Salt content effectively controls the flora distribution (Davis, 1965).

Flavor Development in Cheddar Cheese

Salt in the Development of Typical Cheddar Flavor.

In different lots of Cheddar cheese Irvine (1951, 1955) found a salt content of 0.9-2.9% and reported that low salt cheese more often tended to have unclean flavors and gas holes. The best cheese had about 1.6% salt. Davis (1965) pointed that a cheese made without salt would lose the characteristic flavor and become relatively insipid. Such a cheese would not only lack the inherent salty taste but would acquire quite a different

flavor. Salt takes part in the highly complex phenomena of odor and taste formation of individual cheese varieties. According to Harper (1959), cheese aroma can be divided, through distillation of an acqueous cheese suspension, into two large groups: non-volatiles which consisted of lactic acid, amino acids and others, amines, minerals and sodium chloride and the volatiles which consisted of fatty acids, aldehydes, ketones, alcohols, esters, hydrogen sulfide and other sulfides. In the United States salt ranges of 3.3-6.2% for Provolone and 2.9-9.0% for Romano cheese were found in the dry matter (Harper and Gould, 1952). However, there appeared to be no correlation between salt content and the flavor development in these ranges.

Chemistry of Flavor in Cheddar Cheese.

Kosikowski (1957) stated that the typical flavor of Cheddar cheese is associated with a pleasant, slightly sweet, aromatic, walnutty sensation without any outstanding single component. In aged Cheddar cheese a bitty quality which is neither coarse nor unpleasant gives sharpness to the cheese. The list of compounds isolated from the aroma fraction has become extensive, so it has not been possible to attribute Cheddar flavor to a single or relatively few compounds. Mulder (1952) was the first to advance a theory on a balance of various flavor components responsible for the production of typical Cheddar flavor. His 'Theory of Balanced Components' explained that the typical aroma was due to the combination of many compounds in proper quantitative balance. Kosikowski and Mocquot (1958) proposed another concept called the 'Component Balance Theory' which states that not only for Cheddar flavor but for a range of food flavors, only a small number of compounds are responsible. Thus, for a typical Cheddar flavor the amounts and relative proportions of the flavor

compounds were within certain limits in the cheese.

Each major variety of cheese is different in flavor, body and texture from other cheese varieties and is characterized by the specific kind of ripening it undergoes. Tittsler (1965) attributed the cheese characteristics to the composition, enzyme content, bacterial flora of milk, starter, rennet and other added enzymes, amount of added salt and conditions of manufacturing and curing. The compounds considered to be relatively more important in imparting a typical Cheddar flavor included the volatile fatty acids (Patton, 1963), aldehydes (Keeney and Day, 1957) methyl ketones (Harvey and Walker, 1960), diacetyl (Calbert and Price, 1948), and sulfur compounds (Walker, 1959). Others working in the area of flavor chemistry have reported that compounds like amino acids (Mabbitt, 1955), esters (Dacre, 1955), alcohols (Scarpellino, 1961), partial, glycerides (deMan, 1966), lactic acid (Mulder, 1952), amines (Silverman and Kosikowski, 1956) and peptides (Harwalkar and Elliott, 1965) also probably affect the typical flavor of cheese.

Carbonyl Compounds.

Carbonyl compounds identified in Cheddar cheese have been classified as acidic and neutral. Among acidic carbonyls are oxalacetic, oxalsuccinic, pyruvic, alpha-keto-glutaric, alpha-aceto-lactic and alpha-keto-isocaproic. The neutral carbonyls considered to be the degradation products of acidic compounds (Bassett and Harper, 1956) include diacetyl, butyraldehyde, acetaldehyde, actylmethylcarbinol, acetone, methyl ethyl ketone, 2-butanone, 3-hydroxybutanone, 2-pentanone, 2-heptanone, 2-nonanone, 2-undecanone, 2-tridecanone, formaldehyde, 3-methylthio-propanal (methional), 3-methyl butanal and propionaldehyde. Calbert and Price (1949 a) concluded that the presence of diacetyl in concentrations less

than 1 ppm was necessary in good cheese. A higher amount was associated with flavor defect.

Wolin and Kosikowski (1959) and Wolin (1961) used tritium labelled casein for cheesemaking and found some of the carbonyls in ripened cheese resulted from the degradation of casein. MacLeod and Morgan (1958) reported that the bacteria in cheese form aldehydes from amino acids by transamination and decarboxylation. Schonberg and Moubasher (1952) suggested that the strecker degradation of amino acids may play a part in the formation of Cheddar cheese flavor by production of aldehydes.

Day and Keeney (1958) and Walker and Harvey (1959) have reported that carbonyl compounds are only partly responsible for the Cheddar cheese aroma and suggest that 3-methylthio-propanal, which is produced by strecker degradation of methionine, may be a key component of the Cheddar cheese flavor. In general, Kristoffersen and Gould (1959) found no relationship between the presence and relative concentrations of the individual carbonyl compounds and Cheddar cheese flavor.

Nitrogenous Compounds.

An extensive study of proteolysis in cheese has helped identify several reaction products. Despite certain quantitative differences reported by several workers in the occurrence of these nitrogenous compounds, it is generally agreed that amino acids, amines, and peptide fractions do not impart cheese flavor as such, but appear to function by providing a 'brothy' background on which is superimposed the typical Cheddar flavor. While no direct relationship may be established, a higher flavor score of raw milk cheese over the pasteurized milk cheese is ascribed to the higher amino acid content of raw milk (Day, 1967). Day mentioned that further degradation of amino acids served to introduce a variety of compounds

which were significant for typical Cheddar cheese flavor.

Bitterness in cheese was attributed by Emmons, et al. (1960) to the presence of peptides. Schormuller (1968) showed that bitter taste occurred when certain microorganisms, that participate in cheese ripening and exhibit peptidase activity were missing. Breene, et al. (1964) made cheese by direct acidification with hydrocloric acid and found that a bitter flavor which developed was cured by addition of 1% starter. A low salt concentration always accompanied by a higher moisture content tended to favor development of a bitter flavor in Cheddar cheese (Golding, 1947). Tuckey and Ruehe (1940) associated the production of bitter flavor in Cheddar cheese with undersalting treatment that only incorporated 1.3% or less salt. The effect was not found when the salt values were 1.7% or more. A very slight bitterness is, however, a part of true Cheddar flavor.

Fatty Acids.

Fatty acids originate from fat hydrolysis, metabolism of carbohydrate or degradation of amino acids. The acid spectrum can differ greatly with the type of individual cheese. Peterson, et al. (1949) observed that free fatty acids of intermediate chain length were produced during the ripening of cheese and were of importance in the development of characteristic Cheddar flavor. Mabbit and Zielinska (1956) observed that a typical Cheddar flavor did not develop in skim milk cheese, instead a flavor described as, more like a mixture of amino acids was found. Stadhouders (1956) reported that short chain free fatty acids were desirable in the mixture of fatty acids. Kristoffersen and Gould (1960) found that no cheese flavor developed without appreciable fat hydrolysis.

Patton (1963) studies the volatile fatty acids (acetic, butyric, caproic, and caprylic) in cheese distillates using sodium bicarbonate to block the functional groups of these acids in cheese and cheese distillates. Patton concluded that the volatile fatty acids were the backbone of Cheddar aroma. Acetic acid, a dominant volatile fatty acid was considered to be important. Samples of Cheddar cheese made from raw and pasteurized milks and having a wide range of flavors were analyzed by Bills and Day (1964). They found acetic acid showed the greatest variability in concentration.

Ohren (1965) reported that the typical Cheddar flavor was related to the balance of free fatty acids and acetate. The ratio of free fatty acids to acetic acid was calculated to be between 1:0.55 to 1:1.00 in the finest flavored cheese after 180 days of ripening.

Sulfur Compounds.

Sulfur compounds involved in the formation of cheese flavor are considered to be the degradation products of sulfur containing amino acids of milk, e.g., cystine, methionine, and cystein. Mabbitt (1961) suggested that hydrogen sulfide formation may be an indicator of flavor production and that methyl sulfide may be of more flavor importance. Methyl mercaptan was isolated and identified by Libbey and Day (1963) as a component of cheese flavor. Kristoffersen (1963) and Kristoffersen, et al. (1964) showed that concentrations of hydrogen sulfide and fatty acids are related to the aroma of Cheddar cheese and the concentration of active -SH groups was parallel to the characteristic Cheddar flavor intensity. Sharpe and Franklin (1963) reported that many strains of lactobacilli produced hydrogen sulfide under low pH, anaerobiosis and low sugar concentrations and they may also cause a similar change in cheese.

Other Flavor Compounds.

In addition to those previously mentioned, several other compounds have been isolated and may directly contribute to the flavor or contribute to the formation of other compounds, e.g., ethyl and secondary butyl alcohols (Patton, et al., 1958, Scarpellino and Kosikowski, 1962). The presence and importance of esters of fatty acids and mono hydroxy compounds in cheese were confirmed by Day and Libbey (1964).

CHAPTER III

EXPERIMENTAL

Materials and Methods for Cheesemaking.

Cheddar cheese for this investigation was made from whole milk received from the M.S.U. Dairy farm. A substitute rennet, "Emporase"[a], from culture fermentation of Mucor pusillus var. Lindt, was used for the cheesemaking. A freeze-dried lactic culture[b] was propagated for the manufacture of Cheddar cheese.

The method of manufacturing the unsalted Cheddar cheese was the same as for regular Cheddar cheese except that the salt was not added to a portion of the milled curd. The control cheese contained 1.5% to 1.8% sodium chloride after pressing. A pressure of 60 psig was applied on cheese in 20 lb block hoops and after approximately 2 hr the unsalted lot was removed from the press and kept in cold storage. The hoops of salted cheese were left in the press overnight. The next morning the unsalted cheese blocks were taken out of the hoops, all cheese blocks were then wrapped in a standard cheese aluminum foil coated with waxed paper. Two cheese blocks were sampled, wrapped and coated with wax, in order to prevent moisture loss during curing or storage. Waxing of the exposed area after each subsequent sampling was to prevent moisture loss and mold contamination during ripening. The cheese samples were cured at 45-50°F and approximately 80% humidity in the curing room.

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a. Dairyland Food Laboratories, Waukesha, Wis.
b. Cultures, Inc., Indianapolis, Ind.

Analytical Procedures

In view of the number of the analyses to be made, a slice of approximately 300-400 g was obtained every 2 wk from the unsalted and control cheese blocks. The samples were packaged in polyethylene bags immediately.

The samples were analyzed first for the shear force measurements within a few minutes of sampling. The smaller slices resulting from this experiment were then placed in bottles and stored at -10°F and grated with a food chopper the same day. The well mixed samples were then held frozen at -80 to -90°F if not analyzed the same day.

Measurement of Shear Force.

The Lee-Kramer Recording Shear Press, Model TR-1 was used with the 3,000-pound transducer ring consisting of 10 blades, and No. C-1S standard shear compression cell, to measure the firmness of the samples using the following procedure:

1. After removal of the rind portions, a slice of cheese approximately one-half inch thick, weighing 55^{+}_{-} 1 g was cut from the cheese sample and properly sized to fit into the bottom of the test-cell, without undergoing any deformation.

2. The element was fixed on the transducer ring and the cell-box with the weighed sample under cover was placed into position. Since the temperature of the sample was critical, it was constantly held to 55^{+}_{-} 2 F.

3. For the salted cheese the range of the Shear Press was set at 20, while for the unsalted cheese the setting was 10 except for 1-day old samples, which required a setting of 20. Lower range-settings were made to get emplified peaks.

4. Zero adjustment was made to bring the recorder pen to zero.

5. After the upper shear blades assembly and the lower sample cell were in proper position, the shear blades were passed through the sample which was laying perpendicular to the shear grids of the sample cell, and the resistance to shear was recorded on the strip chart.

6. Readings on each sample of cheese were obtained in triplicate. The maximum peaks were read from the recording chart and were subsequently converted to pounds of shear force per gram of product using the following formula:

$$\text{Pounds force/g sample} = \frac{\frac{\text{Range setting}}{100} \times \text{Peak reading (max.)}}{\text{Sample weight in grams}}$$

Moisture and Total Solids.

Moisture contents of all Cheddar cheese samples were determined by the modified Mojonnier method for moisture in cheese (Milk Industry Foundation, 1952). Moisture content was calculated by the difference in weight of the original sample and the dried solids residue. Determinations were made in duplicate.

Fat.

Fat determination by the Roese-Gottlieb method with Mojonnier modification for cheese was made in triplicate on all samples of cheese (Milk Industry Foundation, 1952).

Salt.

Total chloride contents in the cheese samples were determined by a method of the Association of Official Agricultural Chemists for cheese (AOAC, 1970) and calculated as sodium chloride.

Sodium.

Sodium contents of cheese samples were determined using a Perkin-Elmer 303 Atomic Absorption Spectrophotometer. Homogenous cheese samples were weighed in paper thimbles in 1.5-2.0 g quantities and dried in a vacuum oven overnight. The dried cheese samples were defatted with approximately 30 ml petroleum ether (B.P. 30-60 C) in a Goldfisch Fat Extractor for 7-8 hr and dried again. Samples were transferred to Vicor crucibles, weighed and charred before putting into the muffle furnace for the final ashing at 550°C.

About 4-5 ml of N/10 hydrochloric acid were added to the ash in each crucible and the contents heated mildly for 10 min. Suitable dilutions were made with deionized water to give concentrations within 0.3-3.0 ug sodium/ml. A standard curve was prepared with sodium chloride solutions containing 1, 2, 3, 4, and 5 ug sodium/ml. The method of analysis was essentially that outlined in the Perkin-Elmer handbook (Anonymous, 1968).

Other conditions were as follows:

fuel - acetylene (oxidizing flame) at 10 psig;

oxidizer - air at 40 psig, and

wave length - 295 on the Spectrophotometer (corresponding to 5890 A).

The results were expressed as mg sodium/100 g cheese.

Nitrogen in Total and Water-Soluble Nitrogen Compounds.

Total nitrogen in cheese samples was determined by the semi-micro Kjeldahl method (AOAC, 1970) and the amount of protein calculated by multiplying the amount of total nitrogen by a factor of 6.38.

Using the method of Vakaleris and Price (1959), the amount of nitrogen in water extracts of cheese containing water soluble nitrogenous compounds from protein degradation was determined at weekly intervals. The nitrogen analysis were made singly on duplicate acid-soluble extracts of each cheese sample by the semi-micro Kjeldahl method (AOAC, 1970).

pH.

The pH of the cheese samples were measured on the filtrate obtained by blending 12.5 g well mixed sample of cheese with 17 ml boiled and cooled distilled water and then filtering through a fine muslin cloth. The pH was read to the nearest 0.01 of a pH unit using a glass Calomel electrode.

The dilution of the sample was kept well within the ratio of 1:2 (cheese:water) to prevent any definite shift of the pH reading (Tittsler, 1965).

Free Fatty Acids.

Volatile Fatty Acids.

The rapid direct distillation method reported by Kosikowski and Dahlberg (1946) to recover 100% of the short chain volatile fatty acids, such as, acetic, butyric and caproic, was used. According to these workers, about 90% of the caprylic and a small percentage of capric and lauric acids were recovered by this method. A 10 g sample of cheese drawn every 2 wk during ripening was distilled and the volatile acids in the distillate titrated against N/20 sodium hydroxide solution. Results were expressed in terms of ml N/10 volatile acids in 100 g cheese.

Acetic Acid.

Development of acetic acid in cheese during ripening was measured.

The liquid-liquid partition chromatography procedure of Wiseman and Irvine (1957) as modified by Bills and Day (1964) and Blakely (1970) was used.

A 20 x 300 mm chromatographic tube was filled with a suspension of 15 g Celite in 150 ml Skellysolve B and acetone (1:1 v/v), blended with 2.4 ml of indicator solution and 0.1 ml of N/10 sulfuric acid. Nitrogen pressure (10 psi) was used to pack the adsorbent in the column and expel the liquid until the latter had been expressed to the top of the adsorbent.

A 1% solution of acetone in Skellysolve B (BA_1) was carefully added to the top of the column packing to a depth of about 10 cm. To this was added a slurry of 8 g cap material (sodium sulfate : Celite : ammonium sulfate :: 12 : 8 : 1) in 25 ml of BA_1 solution and the cap was compressed using nitrogen pressure (10 psi). Seventy-five ml of BA_1 solution was passed through the column to wash the acetone-Skellysolve B (1:1 v/v) solution previously used.

The sample mixture was prepared by thoroughly grinding and mixing. Then 5 g of this was acidified to pH 1.8-2.0 (using 50% sulfuric acid) with 10 g of silicic acid in a mortar with a pestle. Two grams of this sample mixture were applied to the top of the column for the acetic acid determination. A filter disc was then placed on top of the column.

Butyric and higher carbon chain fatty acids were removed from the column with 200 ml of BA_1 solution. Acetic acid was eluted with 250 ml of a 15% solution of acetone in Skellysolve B. Titration of the eluted fractions from the column was done in an atmosphere of nitrogen using 0.005 N sodium hydroxide solution. Results were expressed as mg acetic acid per kg of original cheese.

Butyric and Higher Acids.

For the quantitative analysis of individual free fatty acids from butyric through linolenic, the method of Iyer, et al.(1967 a) as modified by Blakely (1970) was used. The silicic acid column of McCarthy and Duthie (1962) was used for the isolation of free fatty acids from cheese. A 5 g representative sample of Cheddar cheese was acidified with a 50% solution of sulfuric acid to a pH of 1.8-2.0. Five to 10 mg each of 7:0 and 17:0 fatty acids were added as internal standards, in about 5 ml of Skellysolve B, to the cheese sample with thorough mixing. Nine grams of silicic acid were also added to the cheese sample and mixed thoroughly.

A 25 x 500 mm chromatographic column was filled with 35 g of previously prepared packing material (Blakely, 1970) slurried in 150 ml of 1% solution of butanol in Skellysolve B. The column was packed using nitrogen at 5-10 psi. The silicic acid-cheese mixture made previously was added to the top of the column packing. The mortar and pestle were thoroughly washed with a 1% solution of butanol in Skellysolve B, and washings returned to the column. Fat was extracted using 400 ml of 1% solution of butanol in Skellysolve B and the eluate saved for the separation of free fatty acids in another column.

A 18 x 200 mm chromatographic column was packed with a mixture of 4 g of specially prepared silicic acid (Blakely, 1970), 8 ml of isopropanol-KOH solution and 24 ml ethyl ether. The column was washed with 100 ml of ether and freed of air bubbles with a glass rod. The eluate from the fat extraction column was passed over this column. The column was then washed with 75 ml of ether to remove the lipids. The free fatty acids were eluted with 60 ml of ether containing 2.5% concentrated

phosphoric acid (v/v).

The eluate from the above column was collected in a 250 ml centrifuge bottle and 70-80 ml of ethanol were added to the eluate. This solution was titrated to the phenolphthalein end-point with 1 N methanolic-KOH solution, under nitrogen. The centrifuge tube was subjected to centrifugation at 1200 rpm for 3-4 min in order to remove the precipitated potassium salts of acidic constituents. The clear supernatant containing soluble potassium salts of the fatty acids was transferred to a 500 ml round bottom flask. The contents of the flask were concentrated to 5-10 ml under vacuum on a rotary evaporator. The 5 ml concentrated sample was quantitatively transferred to a 16 x 125 mm screw cap round bottom test tube and evaporated to dryness at 50°C under a stream of nitrogen. Butyl esters were prepared by adding 0.5 ml of n-butanol, 1 drop of 0.03% methyl red indicator in n-butanol and 0.1 ml of concentrated sulfuric acid into the tube containing the dry salts of the free fatty acids. Butyl esters of free fatty acids were formed by heating the sealed tube in a 100°C water-bath for 1.5 hr. It was removed from the water-bath, cooled and approximately 0.5 g anhydrous sodium sulfate added to the tube.

The butyl alcohol solution was quantitatively transferred to a Babcock skim milk fat-test bottle by washing the tube with 5-10 ml of 1% sodium bicarbonate solution. The esters were brought to the neck of the bottle by adding 20% sodium chloride solution.

The butyl esters of the free fatty acids were analyzed by flame ionization gas chromatography using a Hewlett Packard Research Gas Chromatograph; Series 5750 B, fitted with a disc integrator.

Columns for the gas chromatograph were prepared in a manner outlined

by Dal Nogare and Juvet (1962). A 10 ft by 1/8 in o.d. copper column was packed with 15% diethyl glycol succinate coated on 80/100 mesh acid washed Chromosorb W. Packing was accomplished with the aid of a vibra graver tool. Both columns were matched to insure minimum baseline drift during the temperature programmed analysis of fatty acids esters. After the packing was completed the columns were conditioned in the column oven for 72 hr at the upper limit temperature of 195°C and a nitrogen flow rate of 30 ml/min. After conditioning the columns were attached to the detector system and gas leaks eliminated. The instrument was then ready for the analysis of the fatty acid esters. The following operating conditions were established for gas liquid chromatographic analysis:

injection port temperature, C	270,
detector block temperature, C	280,
hydrogen flow, ml/min.	65,
air flow, ml/min.	500,
nitrogen carrier gas flow, ml/min.	30,
range setting	103,
attenuation, lower limit	1,
column temperature	- programmed 4 C/min from 40°C until first appearance of butanol (solvent), then programmed at the rate of 8 C/min. to 195°C.

Identification of esters was accomplished by their retention times compared to those of the standard butyl esters (obtained from Applied Science Laboratories, Inc., State College, Pa.). Both symetric and asymetric peaks were quantitated using Disc trace according to the method outlined in the 'Series 200, Disc Integrator Manual' (Disc Instruments, Inc., 1969).

Individual fatty acids including the internal standards (heptanoic and heptadecanoic), each in about 10 mg quantity were dissolved in ether, applied to the silicic acid-KOH column and then treated according to the method outlined earlier. The standard fatty acids passed through six different columns were butylated separately and analyzed in triplicate to

obtain the correction factors. These correction factors were obtained for C4:0, C6:0 and C8:0 compared to C7:0 and for C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 compared to C17:0 according to the formulas outlined by Bills, et al., (1963). Use of these factors eliminate the need for precise measurement of the quantity of the sample-injection and permit injection-levels that had desired peak-heights.

Organoleptic Judging.

A small judging panel of 3-5 members of experienced cheese judges scored the cheese. The flavor evaluation was made in a room free from extraneous background odors. Flavor was scored according to the ADSA method. A flavor score of 40 required no criticism. The same score card was used to score the body and texture. The normal range for this score was between 25-30. A score of 30 required no criticism on body and texture. Samples of the salted and unsalted cheese were scored by the judges within an hour after they were drawn from the cheese blocks. Each trial was judged for flavor development every 4 wk.

CHAPTER IV

RESULTS AND DISCUSSION

Composition of Unsalted and Salted Cheddar Cheese.

The summary of chemical analysis of unsalted and salted Cheddar cheese made at the end of the 12 wk curing period appears in Table 1. It shows the average moisture, fat, total solids, total nitrogen, soluble nitrogen, salt and sodium contents of three batches of unsalted and salted Cheddar cheese. Higher moisture content, lower sodium and higher soluble nitrogen were associated with unsalted cheese. The total solids, fat and total nitrogen contents of all salted cheese samples were higher than the unsalted samples. A higher percent of moisture in the unsalted cheese could be partly attributed to the absence of salt, which causes contraction of the curd releasing moisture from protein, (Davis, 1965). The second cause could be the reduced pressing time for the unsalted cheese.

The estimated nitrogen in the soluble nitrogenous compounds hydrolyzed from the insoluble proteins during ripening was found to be higher in the unsalted cheese samples. Again, a higher moisture content and possibly the lack of salt inhibition may account for the higher degree of proteolysis in the unsalted cheese samples.

The addition of salt is primarily important in controlling the growth of undesirable microorganisms, such as, proteolytic bacteria which are particularly sensitive to sodium chloride in the concentrations found in the

Table 1. Comparative analysis of unsalted and salted Cheddar cheese.

Constituents	Lot A		Lot B		Lot C	
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted
1. Fat %	34.77	36.02	32.17	33.92	32.72	35.08
2. Total solids %	59.36	63.90	58.29	62.91	59.76	63.50
3. Moisture %	40.64	36.10	41.31	37.09	40.24	36.50
4. Total nitrogen*)	3572.00	3764.00	3575.00	3840.00	3811.00	3998.00
5. Soluble nitrogen **)	1382.00	805.00	1470.00	1132.00	1370.00	945.00
6. Salt %	0.01	1.79	0.04	1.48	0.03	1.68
7. Sodium, mg/100 g	26.00	704.10	41.50	582.20	53.60	660.80

*) mg nitrogen per 100 g cheese.

**) mg nitrogen in soluble nitrogenous compounds per 100 g cheese at 12 wk.

cheese (Foster, et al., 1957). The unsalted samples from lots A, B, and C contained very low levels of chlorides expressed as sodium chloride. However, these estimations are based on the chloride contents which may come from compounds other than salt. The salts of milk were considered to be chlorides, phosphates and citrates of potassium, sodium, calcium and magnesium (Corbin and Whittier, 1965).

Organoleptic Evaluation of Flavor and Body.

The absence of salt on the development of flavor and body of Cheddar cheese was assessed by an organoleptic evaluation pannel. Evaluations were conducted at 4 wk intervals as the ripening progressed. The data presented in Table 2 reveal that unsalted cheese samples were usually characterized as having off-flavors, such as, bitter, acid, fruity/fermented, flat and unnatural from the first through the third month of ripening. The corresponding salted controls ripened without any serious flavor criticism. A Cheddar flavor developed with advancement of the ripening period. As the ripening of unsalted cheese proceeded, the flavor score definitely improved at 8 wk over the score at 4 wk. However, an overall decline was observed in the flavor score of unsalted cheese at 12 wk, while the controls showed an overall increase in flavor score as ripening advanced. The undesirable flavor of the unsalted cheese could not be related to any one chemical change occurring in the cheese during ripening, except for the bitterness which could be singled out as a dominant flavor.

Salt appears to be a controlling factor in the flavor development of cheese by affecting the kind, extent and direction of physico-chemical changes which occur during ripening. Van Slyke, et al. (1903)

investigated the rate of proteolysis in Cheddar cheese as a measure of ripening and found it increased with a higher moisture content and less salt. Latter, Czulak (1959) ascribed the bitterness in cheese to the high proteolytic activity of rennet at low pH, the accumulation of polypeptides including bitter peptones and the inability of bacterial proteinases at low pH to convert the polypeptides to amino acids.

Compounds responsible for the production of fruity flavor in Cheddar cheese were identified by Bills, et al. (1965), who attributed this defect to the excessive alcohol production and to the subsequent formation of ethyl esters of free fatty acids. In general, the presence of compounds imparting unnatural and undesirable flavors in cheese could be due to the new balance of microflora and the changed environment within the cheese, such as lower pH, higher moisture content, lower osmotic pressure, and other related physico-chemical changes associated with low salt concentration.

Salt by itself also has a strong masking effect on the off flavors. Harper (1959) divided cheese flavor into the non-volatile and volatile components. He reported that the non-volatile constituents, which include lactic acid, amino acids, amines, minerals and common salt influenced the flavor. Salt masked some of the undesirable flavors of cheese and improved the overall flavor scores. This was demonstrated by a separate set of experiments during this investigation. The data in Table 3 are in agreement with that published by Harper (1959). A panel of four cheese judges was served with cheese after 6, 12 and 24 wk of curing. The unsalted cheese was blended with 0.5%, 1.0% and 1.5% sodium chloride. The control with 1.48% salt was cured for the same periods. Blended cheese flavor ranged from 35.75 to 37.75. As the percentage of

Table 2. Comparison of body and flavor scores of unsalted and salted Cheddar cheese.

Lot no.	Average flavor score	Remarks	Average body score	Remarks
		4 weeks		
A. Unsalted	35.50	Bitter, flat, unnatural, acid, whey taint.	26.50	Pasty, weak, mealy.
Salted	39.00	None.	28.00	Curdy, corky, short, mealy.
B. Unsalted	35.33	Bitter, acid, unnatural, flat.	26.00	Pasty, weak, mealy.
Salted	38.16	Flat, heated, whey taint.	27.50	Corky, crumbly, short, mealy.
C. Unsalted	37.62	Acid, flat, whey, taint.	27.12	Gassy, open, pasty, weak, mealy.
Salted	39.12	Falt.	28.62	Corky, curdy, short, mealy.
		8 weeks		
A. Unsalted	37.33	Flat, unnatural, acid, sl. bitter.	26.33	Gassy, pasty, weak, open.
Salted	39.00	Sl. flat, heated.	28.83	Curdy.
B. Unsalted	36.50	Flat, unnatural, acid, bitter.	26.33	Gassy, open, weak.
Salted	38.33	Acid, flat.	28.66	Open, short.
C. Unsalted	37.00	Acid, fermented, flat, bitter.	26.87	Pasty, gassy, open, weak.
Salted	39.50	Flat.	29.25	Corky.
		12 weeks		
A. Unsalted	36.37	Flat, fermented, bitter.	26.87	Pasty, open, gassy, weak.
Salted	39.50	Flat.	29.37	Corky, open, weak.
B. Unsalted	36.37	Flat, fermented, bitter,	27.12	Pasty, open, weak.
Salted	39.25	Acid, flat.	29.37	Open.
C. Unsalted	37.20	Acid, bitter, whey taint, sl. flat, fruity.	27.30	Pasty, open, weak.
Salted	39.20	Fermented, flat.	29.00	Curdy, open, sl. curdy.

The flavor and body scores represent the average of 3 to 5 trained dairy products judges.

salt increased from 0.0 to 1.0, usually the overall flavor score increased. However, the addition of 1.5% salt did not necessarily improve the flavor over 1.0%. At 6 wk curing the flavor score of cheese ripened without salt but scored after blending with 1.0-1.5% salt surpassed the flavor score of the corresponding salted controls. This may be explained by the more rapid curing of the unsalted cheese, the presence of flavor compounds expected in salted cheese after a longer curing period and the masking of undesirable flavors by the incorporation of salt.

The development of body and texture characteristics is comparatively a quicker change in unsalted cheese than that of the flavor. In all organoleptic evaluation conducted at the end of 4, 8 or 12 wk, the unsalted cheese was criticized as having a weak, pasty, open, mealy or gassy body (Table 2). The increase in the protein hydrolysis and higher moisture may explain the weak and pasty body of unsalted cheese. The photograph (Fig. 1) taken of a cross-section of two lots of 4-5 wk old salted and unsalted cheese illustrates the conspicuous body and textural differences. While some of the openings in the unsalted cheese may be gas holes, most of them appear to be mechanical openings resulting from incomplete fusion of the curd during pressing. Another explanation is that the unsalted curd chunks after milling do not undergo the physical shrinkage from the addition of salt, so when placed in the hoop and pressed, the outer mass of the curd in the hoop immediately yields to the pressure applied in the press. As a result of this, a firm outer layer is formed providing a barrier against the elimination of moisture and air during pressing.

In an anaerobic environment and acidic medium, characteristic of ripening Cheddar cheese, Platt and Foster (1958) showed that homofermentative streptococci produced significant amounts of acetic acid, carbon

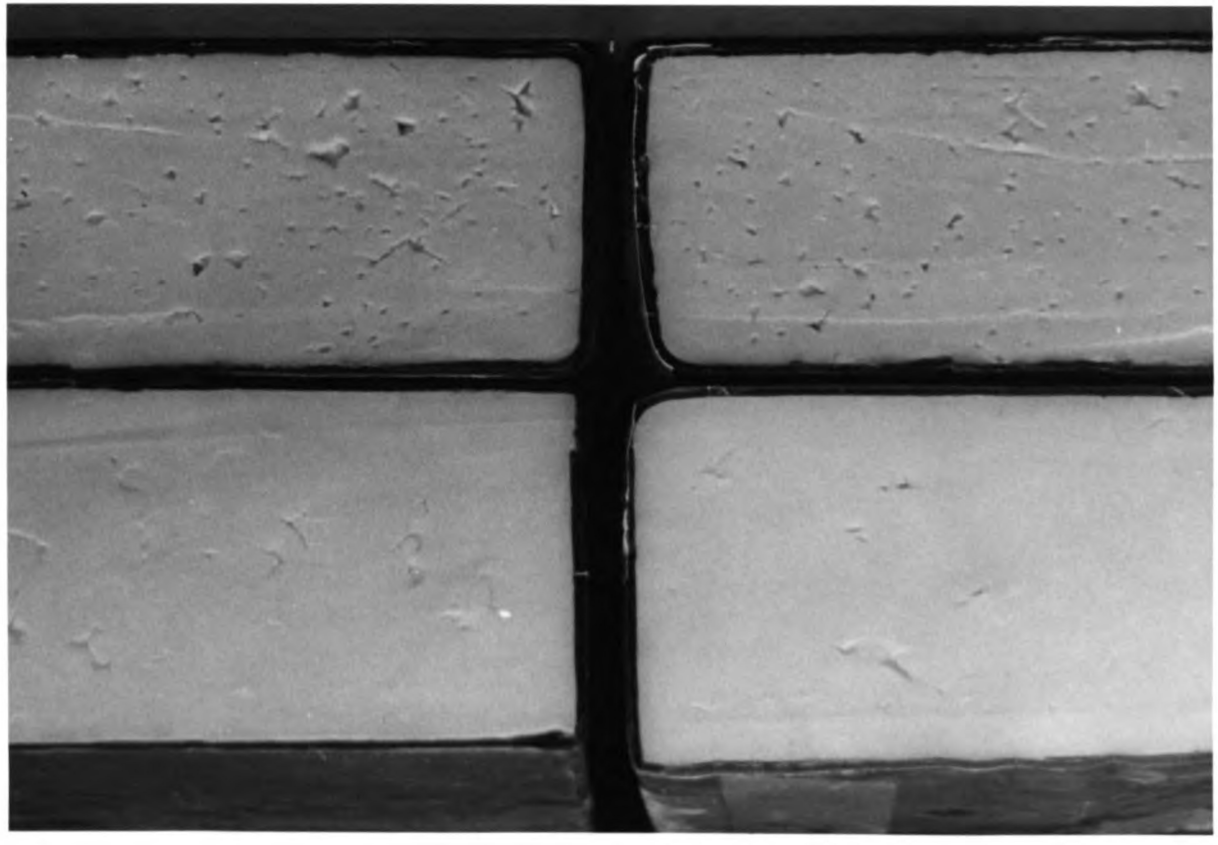

Figure 1. Body and texture of Cheddar cheese cured for 4-5 wk. (Top) Unsalted, (Bottom) Salted.

Table 3. Flavor evaluation of ripened unsalted Cheddar cheese with different amounts of salt added before judging.

% salt added	Average flavor scores		
	6 wk	12 wk	24 wk
0.00	37.00	34.62	36.00
0.50	36.88	35.75	36.50
1.00	37.75	36.10	37.25
1.50	37.12	36.87	37.00
Control: 1.48	37.12	37.12	37.37

The flavor scores are the average given by a panel of four trained dairy products judges.

dioxide and ethanol, in addition to lactic acid. Bang (1949) also noted that degradation of milk sugar by S. lactis can take place both homo- and heterofermentatively, the latter occurring at lower temperatures (59-60°F). In the initial stages of ripening, bacterial population chiefly consists of lactic streptococci, outgrowing other microbial flora. Therefore, production of gas in unsalted cheese, would not be surprising because of the demonstrated ability of the starter bacteria to produce carbon dioxide in the existing environment. Irvine (1951, 1955) also reported after an extensive survey of cheese that low-salt cheese had a tendency to have unclean flavors and gas holes.

Firmness Evaluation Using Shear Press.

To obtain a more precise evaluation of the body of the unsalted and salted Cheddar cheese, the Lee-Kramer Recording Shear Press, Model TR-1

was used with the 3,000 lb test-ring and No. C-1S standard shear compression cell. The firmness was evaluated by measuring the extent of deformation of the proving ring from the force required to compress and shear the sample in the test cell. The compression-shearing action of the standard cell thus simulates the action of a tooth in the chewing of food (Anonymous, 1967). Table 4 presents comparative data on the shear force expressed as $lb_{f/g}$ sample, for each pair of unsalted and salted Cheddar cheese obtained over the 12 wk ripening period. The results indicate that the shear force values of salted cheese appreciably increased in the 1,2 and 4 wk of ripening and then declined to 10 wk. No increase was found in the unsalted cheese as the ripening advanced, with one exception. Generally, unsalted cheese samples decreased very slightly in the shear force as the ripening advanced after 2 wk (Fig. 2). The curves have been shown with standard deviations, which are comparatively smaller for the unsalted samples than for the salted controls. However, the shear force measurement for unsalted cheese at all stages of ripening were found to be lower than those for the controls. In all trials of salted and unsalted cheese samples, the ratio of shear force (salted/unsalted) was minimum for the 1-day old cheese and increased to the 4 wk. At 6 wk the value declined until 10 wk. The increase in the ratio was attributed to the increase in the shear force values of salted cheese and decrease in those of unsalted cheese. The ratio had a wide range of variation, the minimum being 1:1.8686 on the first day and maximum 1:3.0425, at the end of 4 wk. Foster, et al., (1957) reported that solubilization of protein and the high moisture content of the cheese are the important factors responsible for the softness of the cheese. This finding supports the results obtained in this investigation

Table 4. Comparison of the average shear force in $lb_{f/g}$ cheese during ripening.

Age of cheese	Average shear force in $lb_{f/g}$ sample of cheese *						Mean (Unsalted)	Mean (Salted)	Shear force (Salted) / Shear force (Unsalted)
	Lot A		Lot B		Lot C				
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted			
1 day	0.1016	0.2642	0.1060	0.2314	0.1832	0.2343	0.1302	0.2433	1:1.8686
2 wk	0.1257	0.3599	0.1065	0.3194	0.1056	0.2453	0.1126	0.3082	1:2.7371
4 wk	0.1015	0.3532	0.1009	0.3199	0.1147	0.2919	0.1057	0.3216	1:3.0425
6 wk	0.0964	0.3569	0.1017	0.2815	0.1069	0.2471	0.1016	0.2951	1:2.9045
8 wk	0.0918	0.3280	0.0981	0.2540	0.0976	0.2536	0.0958	0.2785	1:2.9070
10 wk	0.0883	0.2781	0.0851	0.2482	0.0936	0.2248	0.0890	0.2503	1:2.8123
12 wk	0.0916	0.2778	0.0957	0.2778	0.0802	0.2046	0.0891	0.2534	1:2.8439

* The data are the average of triplicate tests.

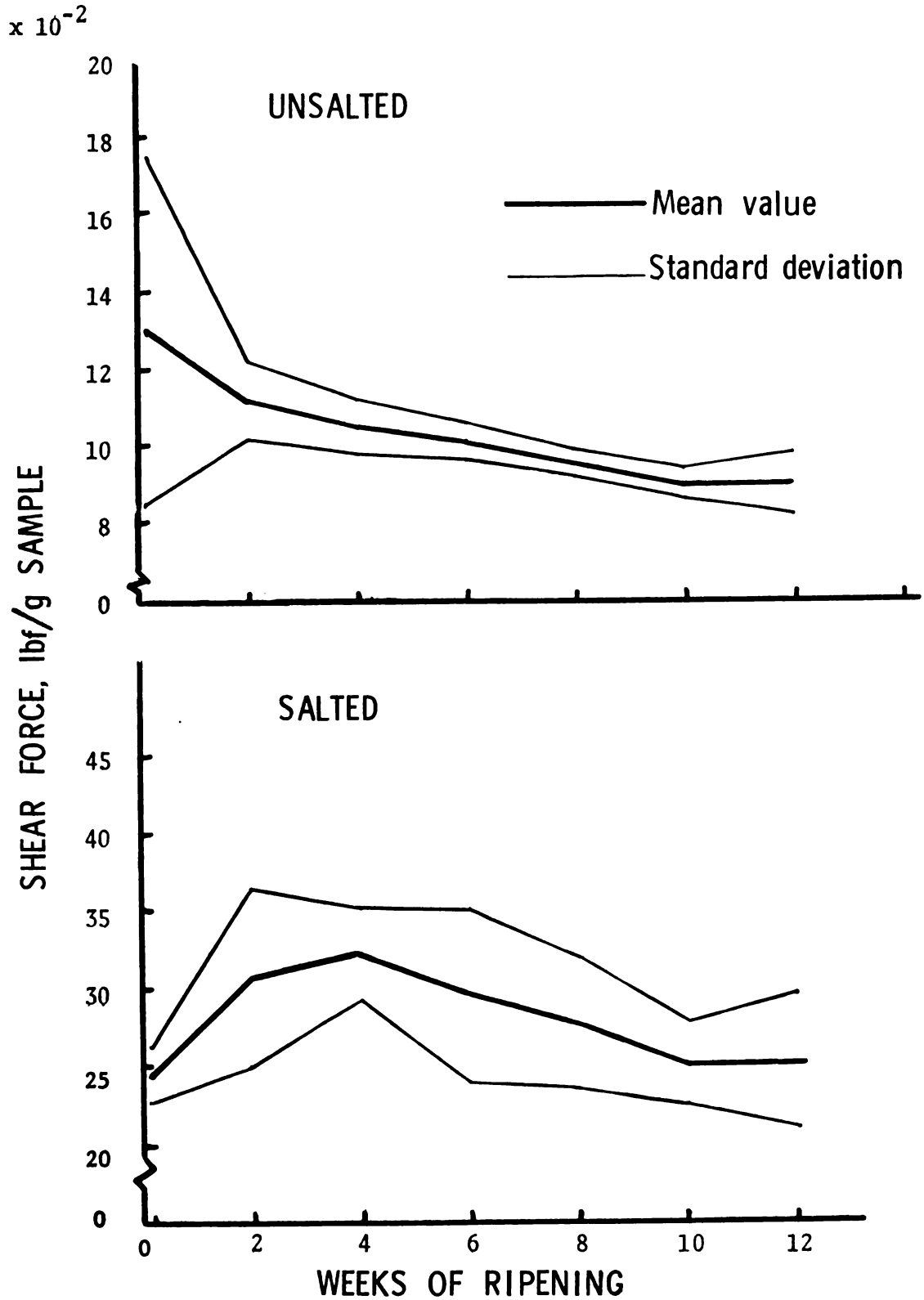

Figure 2. Changes in shear force in unsalted and salted Cheddar cheese during ripening.

and explains why the unsalted cheese had a lower shear force than the salted controls.

According to Van Slyke (1928), the form in which the protein exists affects the elasticity of the curd and therefore, affects the characteristics of the ripened cheese. A green cheese is spongy and curdy. As it ripens it becomes soft and mellow. Van Slyke (1928) theorized that casein in a rennet curd cheese exists as dicalcium paracaseinate and when lactic acid is formed by the starter organisms, it reacts with the paracaseinate as follows:

1. dicalcium paracaseinate + lactic acid $\longrightarrow$ monocalcium paracaseinate + calsium lactate;
2. monocalcium paracaseinate + lactic acid $\longrightarrow$ free paracasein + calcium lactate.

Free paracasein and its monocalcium salt have widely different physical properties. As lactic acid is produced in appreciable amounts, cheese curd begins to show elasticity. Van Slyke (1928) further attributed the elastic and ductile properties of cheese curd to the presence of monocalcium paracaseinate (reaction 1). He demonstrated that this compound comprises over 90% of the nitrogenous materials in the cheese a few hours after the curd is put into the press. As the acid production continues, the curd loses much of its elasticity, becoming hard and brittle, probably due to the formation of free paracasein (reaction 2). Paracasein does not show the elastic properties of its monocalcium salt. The formation of free paracasein may account for the increase in the shear force values of salted cheese at the start of the ripening. In the case of unsalted cheese, this effect is probably counteracted by the increased moisture of the cheese causing the lower shear force values in these samples. A report from Sazabo (1966) supports this inference

by stating that the viscosity and elasticity of the product are lowered if its moisture content or fat content is raised. Sohngen, et al. (1937) attributed the physical changes in the curd to viscosity and the degree of hydration of casein. While studying the rheological changes occurring in the small brick Dutch-type cheese during the first 3 wk of ripening, Kunakhov (1967) reported that the maximum shearing stress increased immediately after the pressing and the relative residual deformation decreased to 1/5 of fresh cheese by the 10th day.

Thus, comparatively lower shear force values for all samples of unsalted Cheddar cheese at various stages of ripening may be ascribed to a high moisture content, extensive solubilization of protein and possibly to the mechanical openings and the gas holes present in the cheese.

Protein Degradation Changes.

During cheese ripening, protein is one of the major milk constituents undergoing physical and chemical changes. The extent of proteolytic action and specific compounds resulting there from help to determine the characteristics of the final cheese. Foster, et al. (1957) stated that some of the products of protein decomposition contribute to the flavor. Peptones in general, are bitter and they may account in part, for the background flavor. Certain of the amino acids and simple peptides have considerable influence on taste.

The enzymes responsible for the proteolytic decomposition in cheese primarily originate from rennet or other added natural enzyme preparation and microorganisms. Rennet, which is used in the manufacture of most types of cheese, has a primary function to coagulate casein, but its proteolytic action also continues during the ripening period (Harper

and Kristoffersen, 1956).

Practically all the nitrogenous constituents of uncured cheese exist as water insoluble protein, but as the ripening progresses, part or all of the protein is hydrolyzed enzymatically to simpler components that are water soluble. The general course of these changes may be illustrated as:

Protein ⟶ Proteoses ⟶ Peptones ⟶ Peptides ⟶ Amino acids.

In the above scheme, all but the protein are water soluble.

Among its several functions in cheese, salt helps control moisture and acidity development. Of primary importance is the function of salt in controlling growth of undesirable microorganisms. Strongly proteolytic bacteria, for example, are sensitive to sodium chloride in concentrations found in most cheeses (Foster, et al., 1957). Davis, et al. (1937) indicate that, of a number of chemical substances added to Cheddar cheese, salt exerted the largest effect. Omission of salt resulted in a 50% increase in ripening rate as measured by protein breakdown.

If moisture is mechanically retained in a normal salted cheese (by waxing), an increased rate of protein breakdown is obtained (Van Slyke and Price, 1949). It is, therefore, difficult to calculate the specific effects of salt concentration and moisture separately.

The decomposition of protein in the cheese can be followed in any of several ways, the most common method being the determination of the amount of nitrogen in the water extracts of cheese at different ages. As protein is hydrolyzed to water soluble compounds, the value increases, showing the progress of ripening. In this experiment, all nitrogen analyses were made on duplicate acid-soluble extracts of each cheese by

the procedures essentially described by Vakaleris and Price (1959). The experimental data presented in Table 5 indicate a progressive solubilization of milk proteins both in unsalted and salted Cheddar cheese samples throughout the 12 wk ripening. In cheese curd held at 50°F the 'water-soluble' nitrogen expressed as per cent of total nitrogen in cheese increased from the range of about 4.60-7.15% to a maximum of 21.4-29.5% in the salted cheese, compared to 6.0-8.0% to a maximum of 36.0-41.1% in the unsalted cheese during 12 wk ripening. The curves in Figure 3 present the mean 'water-soluble' nitrogen values and standard deviations of three different batches of unsalted and salted Cheddar cheese.

Acid Changes During Ripening

pH of Cheese During Ripening.

Since pH influences the activity of enzyme systems involved in the ripening (Morris and Jezeski, 1953), the change of pH in the cheese made with salt and without salt was determined. Baribo and Foster (1951) stated that the apparent influence of pH in the cheese might be explained by the effect on the proteolytic activity of rennin. The lower the pH the greater the activity that occurs. Foster, et al. (1957) reported that the rate of acid formation increased rapidly, because the majority of organisms were concentrated in the curd rather than lost with the whey. Unsalted cheese because of its relatively higher retention of lactose provides more substrate for the formation of acid, which in turn, lowers the pH. The starter organisms continue to grow and produce acid using lactose as their substrate. This may account for the initial decline in the pH of cheese when the ripening starts. The unsalted cheese had a lower pH during ripening than the salted cheese. The data on pH

Table 5. Comparison of the amounts of nitrogen in the soluble nitrogenous compounds present in the samples of unsalted and salted Cheddar cheese during ripening.

Sampling interval	'Soluble nitrogen', mg per 100 g of Cheddar cheese.							
	Lot A		Lot B		Lot C		Mean	
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted
1 day	245.0	227.5	285.8	274.2	227.5	186.7	252.8	229.5
1 wk	431.6	268.3	618.3	455.0	466.6	326.7	505.5	350.0
2 wk	665.0	284.6	665.0	484.2	700.0	443.3	676.7	404.0
3 wk	694.2	425.8	898.3	688.3	793.0	490.0	795.2	534.7
4 wk	968.3	553.3	991.6	775.8	968.3	554.1	976.1	627.6
5 wk	984.0	571.7	1015.0	822.5	1020.8	653.3	1006.6	682.5
6 wk	1096.6	641.6	1160.0	857.5	1073.3	676.7	1110.1	725.2
7 wk	1131.6	723.3	1236.6	910.0	1190.0	711.7	1186.0	781.4
8 wk	1225.0	739.6	1300.8	980.0	1295.0	758.3	1273.6	826.0
9 wk	1248.3	752.5	1376.7	1073.3	1330.0	770.0	1318.3	865.2
10 wk	1341.4	764.2	1423.3	1102.5	1359.2	898.3	1374.6	921.7
11 wk	1370.8	793.3	1458.3	1120.0	1365.0	933.3	1398.0	948.8
12 wk	1382.5	805.0	1470.0	1131.7	1370.8	945.0	1407.8	960.6

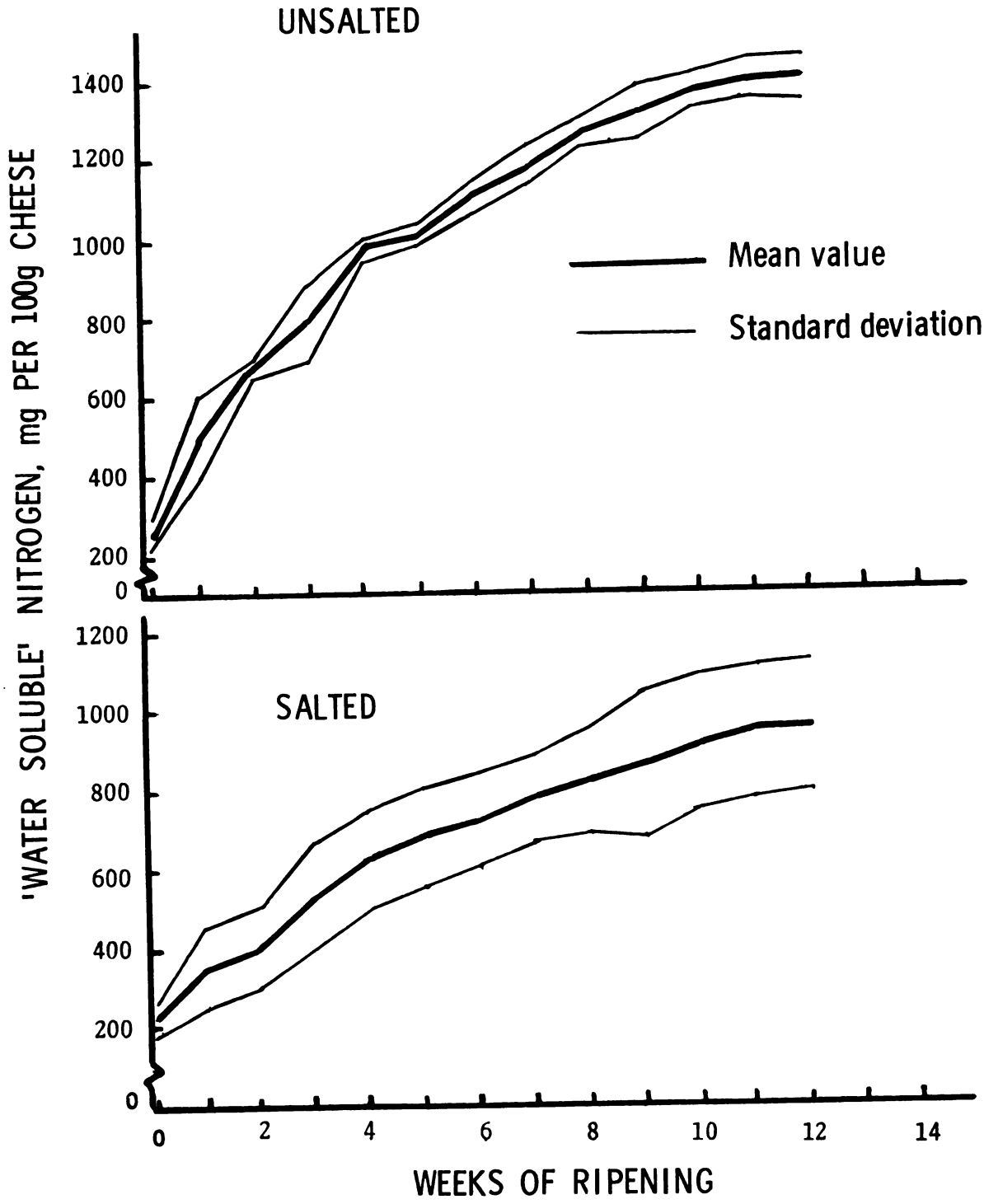

Figure 3. Changes in 'water soluble' nitrogen of unsalted and salted Cheddar cheese during ripening.

during this investigation of 12 wk ripening (Table 6) are indicative. The pH values of salted cheese decreased in two of the three lots during the first week of ripening. Then small erratic results followed. Figure 4 shows the mean pH values and standard deviations for three different batches of unsalted and salted cheese at weekly intervals of ripening.

Table 6. Comparison of pH on samples of unsalted and salted cheese during ripening.

Sampling interval	pH of cheese							
	Lot A		Lot B		Lot C		Mean	
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted
1 day	5.30	5.52	5.23	5.36	5.14	5.56	5.22	5.48
1 wk	5.18	5.41	5.07	5.23	5.23	5.75	5.16	5.46
2 wk	5.19	5.59	5.08	5.27	5.24	5.63	5.17	5.49
3 wk	5.22	5.54	5.12	5.26	5.24	5.69	5.19	5.49
4 wk	5.21	5.52	5.18	5.39	5.24	5.67	5.21	5.52
5 wk	5.27	5.70	5.22	5.34	5.26	5.69	5.25	5.57
6 wk	5.31	5.64	5.22	5.40	5.28	5.60	5.27	5.54
7 wk	5.38	5.71	5.23	5.37	5.27	5.66	5.29	5.58
8 wk	5.34	5.65	5.21	5.42	5.24	5.63	5.26	5.56
9 wk	5.34	5.73	5.27	5.32	5.24	5.81	5.28	5.62
10 wk	5.32	5.72	5.24	5.44	5.25	5.65	5.27	5.60
11 wk	5.33	5.79	5.28	5.45	5.27	5.76	5.29	5.66
12 wk	5.35	5.81	5.28	5.46	5.21	5.52	5.28	5.59

The data are the average of duplicate trials.

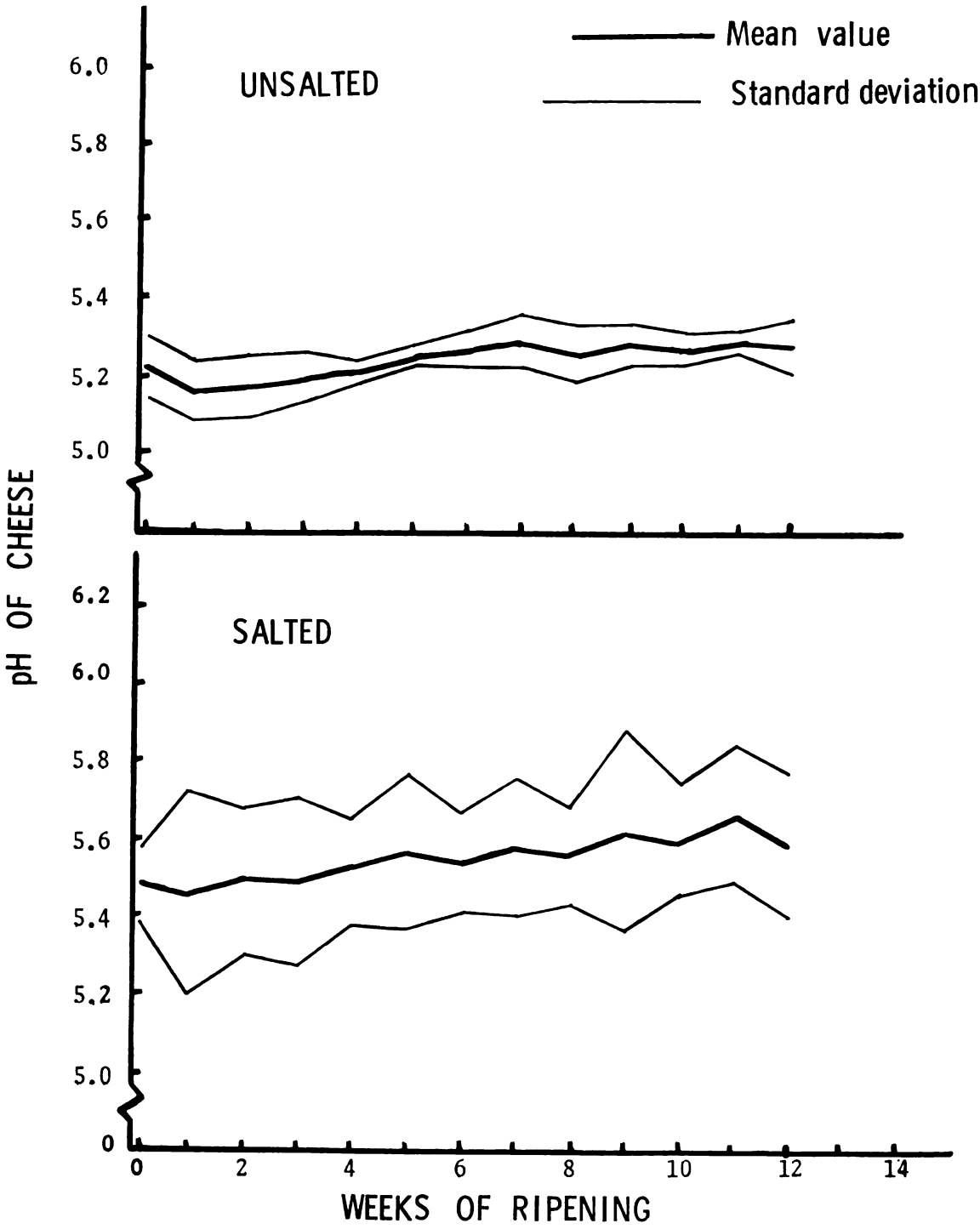

Figure 4. Changes in pH of unsalted and salted Cheddar cheese during ripening.

The chemical reactions that occur during ripening of Cheddar cheese cause a slow but steady increase in the pH according to Brown and Price (1934), who stated that a good quality salted cheese should exhibit this trend in the pH. As indicated by the pH curves in Figure 4, both unsalted and salted cheese showed the same general trend in pH development. The gradual increase in pH is caused by (1) the destruction of lactic acid, (2) the formation of non-acidic decomposition products, (3) production of less highly dissociated acids including acetic and carbonic acids, and (4) liberation of alkaline products of protein decomposition (Tittsler, 1965). Part of this change could also be ascribed to utilization by the organisms of lactic and fatty acids as nutrients. (Foster, et al., 1957).

Volatile Fatty Acids.

Foster, et al. (1957) reported an increase in the volatile fatty acids, acetic, n-butyric, caproic, caprylic and capric during the ripening of Cheddar cheese. Although, not as extensive in its decomposition as protein, the fat of cheese also undergoes a certain amount of hydrolysis during ripening. Petersen, et al.(1949) reported that a part of n-butyric, caproic, caprylic and capric acids likely resulted from hydrolysis of milk fat. Lactose fermentation also may produce lactic acid, acetic acid and n-butyric acid during early stages of ripening. The changes in volatile fatty acids which have been investigated relate the changes which occur between the time of manufacture and the normal time of removing the cheese from the curing room. No investigations have been made during storage.

The mean volatile fatty acids of three batches of unsalted and

salted Cheddar cheese are shown in Table 7. The curves with the standard deviations appear in Figure 5. From the first day to the second week of ripening, the volatile fatty acids showed maximum increase in both the unsalted and salted cheese. The average increase in the volatile fatty acids from 22.90-24.58 ml of N/10 acid per 100 g unsalted cheese occurred while the corresponding increase for the salted cheese was from 16.70 ml for 1-day old cheese to 19.98 ml of N/10 volatile fatty acids at the end of 2 wk ripening. Until the 8 wk of ripening, the mean volatile fatty acids of both the unsalted and salted cheese increased. During the subsequent period of ripening, a slow but gradual increase was observed in the unsalted cheese, whereas small fluctuations occurred in the salted cheese. In all samples, the average values for unsalted cheese remained higher than the corresponding values for the salted samples.

While the higher chain volatile fatty acids, caproic, caprylic and capric, seem to be characteristic of the mold-ripened cheeses, they are also characteristic of the later stages of ripening of Cheddar cheese (Suzuki, et al. 1910; Peterson, et al. 1949). Volatile fatty acids in cheese consists of acetic and other lower carbon chain fatty acids. An examination of acetic acid and lower carbon chain fatty acids in the unsalted and salted cheese samples revealed that a higher volatile fatty acids level in the unsalted cheese as compared to that in the controls was due to a higher acetic acid content in the unsalted cheese.

Acetic Acid.

The per cent recovery for acetic acid was determined in order to calculate its true amount in the cheese samples. The liquid-liquid partition chromatography of Wiseman and Irvine (1957) was used with

Table 7. Quantitative changes in volatile fatty acids of Cheddar cheese during ripening.

Age of cheese	ml of N/10 volatile fatty acids per 100 g cheese.						Mean (Unsalted)	Mean (Salted)
	Lot A		Lot B		Lot C			
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted		
1 day	24.40	20.25	22.05	18.60	22.25	11.25	22.90	16.70
2 wk	24.75	21.05	24.00	23.00	25.00	15.91	24.58	19.98
4 wk	25.75	24.16	26.05	23.80	25.30	16.10	25.70	21.35
6 wk	26.50	25.00	26.60	24.90	25.50	17.00	26.20	22.30
8 wk	26.75	23.40	27.62	26.00	26.50	18.50	26.95	22.63
10 wk	29.50	23.25	28.00	24.12	26.37	18.00	27.95	21.79
12 wk	25.00	21.87	25.12	23.50	27.50	18.70	28.87	21.35
14 wk	28.25	22.50	27.35	24.10	28.50	21.35	28.03	22.65

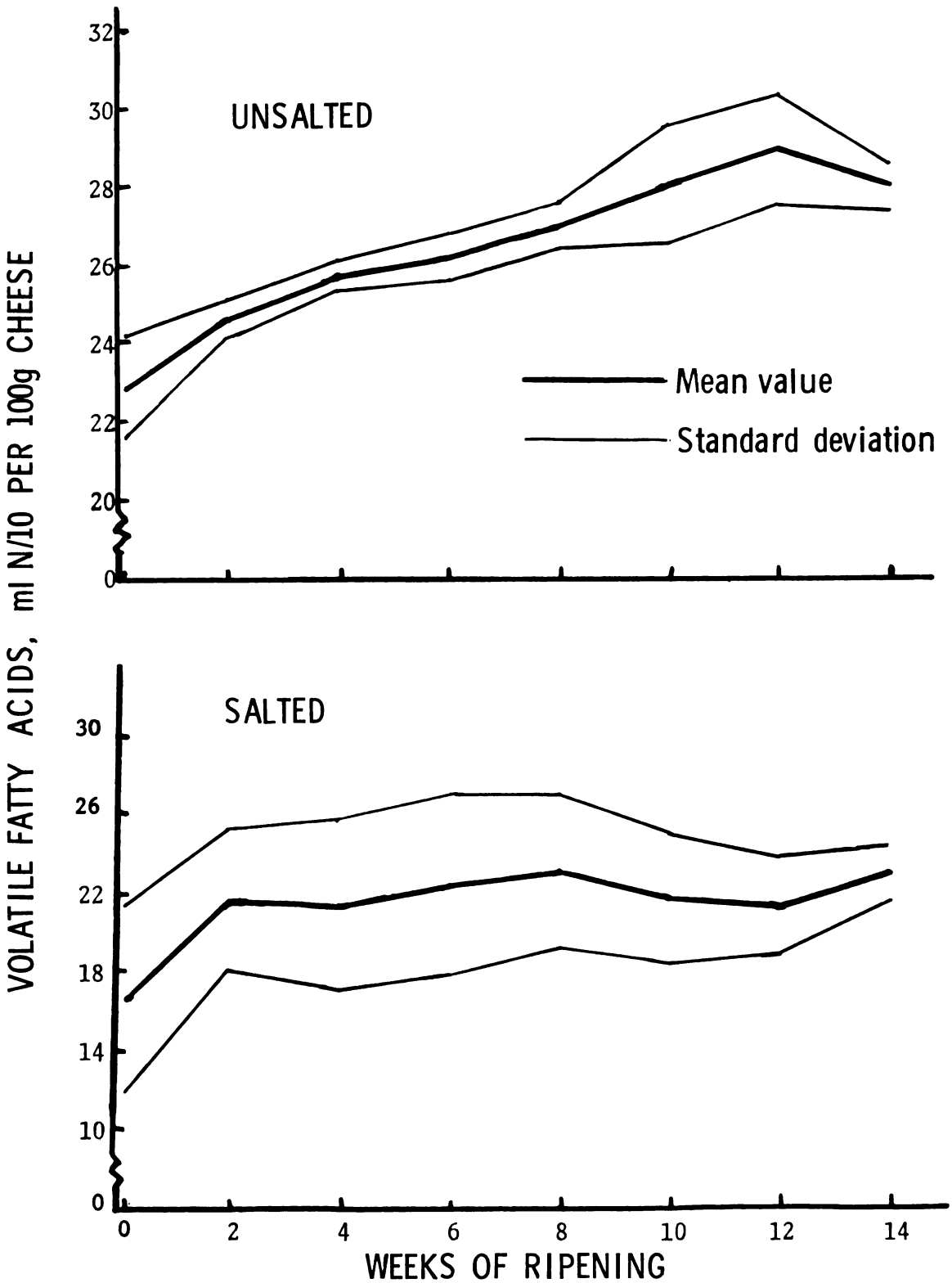

Figure 5. Changes in volatile fatty acids in the unsalted and salted Cheddar cheese during ripening.

the modifications of Blakely (1970). Concentrations of acetic, propionic and butyric acid in water were used for the recovery mixture using the method described in the experimental section. The percentage recovery of the acetic acid was found to be 98.7 and was used as the correction factor for the results obtained on the samples.

Average acetic acid content in the samples of unsalted cheese increased steadily during 10 wk of ripening. At the end of 12 wk a slight decrease was observed. Salted controls generally showed a gradual increase in the acetic acid content up to 8 wk. Variations were observed for the 10, 12 and 14 wk analysis. The acetic acid in the unsalted cheese was higher than in the salted controls during ripening. These data on acetic acid in Cheddar cheese during ripening do not agree with the results of Peterson, et al. (1949). They showed butyric acid to be much higher than acetic acid even on the zero day and observed no appreciable change in the acetic acid content throughout a ripening period of 420 days. Throughout this investigation, acetic acid was the predominant acid in unsalted as well as the salted cheese. Similar findings were observed by Berridge, et al. (1953), Windlan (1955) and Ohren (1965).

Butyric and Higher Fatty Acids.

The method described by Iyer, et al. (1957, a, b) as modified by Blakely (1970) was used for isolation and quantitation of free fatty acids from butyrate through linolenate in cheese samples. Recovery factors were determined and are shown in Table 9.

As appears in Table 10, there was a marked difference in the free fatty acid contents of the unsalted and salted cheese samples, although qualitative results showed that identical fatty acids were liberated

Table 8. Acetic acid contents of unsalted and salted Cheddar cheese during ripening.

Sampling interval	Acetic acid, mg per 1,000 g of cheese.						Mean (unsalted)	Mean (salted)
	Lot A		Lot B		Lot C			
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted		
1 day	458.4	402.2	407.9	285.4	417.3	169.5	427.8	285.7
2 wk	433.1	349.1	414.5	368.1	461.2	212.7	436.2	310.0
4 wk	454.4	425.5	424.8	378.2	479.1	199.0	452.8	334.2
6 wk	532.0	487.3	459.9	369.2	449.1	238.9	480.3	365.1
8 wk	520.3	465.3	468.1	466.7	499.7	226.5	496.0	386.1
10 wk	476.3	392.6	563.5	422.1	506.5	285.5	515.5	366.7
12 wk	498.3	417.3	525.7	410.4	507.9	282.8	510.7	336.9
14 wk	532.6	402.2	516.2	450.3	514.1	295.8	521.0	382.8

in all lots of cheese. All even-numbered carbon fatty acids from butyric to linolenic were found in each lot of cheese throughout the 12 wk ripening period. The concentrations of specific fatty acids varied among the three batches but, in all cases, myristic, palmitic, stearic and oleic acids were present in relatively greater concentrations than the other fatty acids. This was true throughout the ripening period for both unsalted and salted cheese. During this investigation, it was noted that some of the fatty acids were at maximum concentration at 4 wk of ripening and then decreased, while others increased to the 8 wk of ripening before a decrease was observed. Some fatty acids showed variations in concentration as the ripening progressed. In most cases the fatty acids higher than capric had a tendency to increase for the first 8 wk of ripening and then to decrease toward the end of 12 wk. Linolenic acid was relatively low in salted as well as unsalted cheese. Ohren (1965) found a similar trend in the development of free fatty acids in Cheddar cheese. He observed an increase in these acids toward the end of a 6 mo. ripening period, although a decrease in several free fatty acids at 8 wk and 12 wk ripening period was also observed. 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 (Foster, et al., 1957).

Peterson, et al. (1948) demonstrated that the reason for an increase in fatty acids in Cheddar cheese was because lipases became more active in young Cheddar cheese after 5-10 days of ripening. These lipases may represent intracellular enzymes of lactic acid bacteria, liberated by bacterial autolysis. They also reported that part of free n-butyric acid and all of free caproic, caprylic and capric acids

present in 420 day old cheese were the result of the action of these liberated intracellular bacterial lipases on the milk fat in the cheese. Ohren (1965) found that the microflora in the milk and cheese had more influence in promoting lipolysis in Cheddar cheese than normal milk lipases. He observed that increasing fat hydrolysis was accompanied by an increase in acetic acid production. Although it is well known that lipolysis takes place in Cheddar cheese, the source of lipase enzyme has been a matter of controversy. The mechanism proposed for the lipase reaction on milk fat results in the following mixture of compounds:

triglyceride + lipase $\longrightarrow$ triglyceride + diglycerides + monoglycerides + glycerol + fatty acids.

In order to further investigate the role of various factors in the lipolysis of fat in the Cheddar cheese, Ohren (1965) studied the effects of lactic acid, rennet, starter organisms and microorganisms isolated from Cheddar cheese. He found that lactic acid and rennet did not effect an increase in the fat hydrolysis over a period of 49 days. However, starter organisms, as well as, microorganisms isolated from cheese exhibited lipolytic activity when grown in sterile milk. He also discovered that a micrococcus organism isolated from cheese was lipolytic in sterile milk. This evidence, in part, may explain the increased level of fatty acids in the samples of unsalted cheese. Salt has an inhibitory effect on the bacterial population in the medium. Therefore, unsalted cheese is expected to have a much higher population of ripening organisms, which may liberate lipase through autolysis. Gaffney and Harper (1965) emphasized that the lipolysis in cheese may not be due to one type of organism, but the total lipolysis may be due to an additive effect of

Table 9. Correction factors for calculating the actual amounts of butyl esters of fatty acids in relation to the internal standards (heptanoic and heptadecanoic acids).

Acids	Number of trials	Mean correction factor	Standard deviation
4:0 (Butyric)	6	0.88843	±0.0137
6:0 (Caproic)	6	0.88133	±0.0273
7:0 (Heptanoic)	6	1.00000	---
8:0 (Caprylic)	6	0.86085	±0.0146
10:0 (Capric)	6	0.72803	±0.0468
12:0 (Lauric)	6	0.77188	±0.0197
14:0 (Myristic)	8	1.03510	±0.0409
16:0 (Palmitic)	6	0.87546	±0.0255
17:0 (Heptadecanoic)	6	1.00000	---
18:0 (Stearic)	6	1.15115	±0.0342
18:1 (Oleic)	5	0.93360	±0.0602
18:2 (Linoleic)	5	1.98840	±0.0868
18:3 (Linolenic)	5	1.11180	±0.0308

different microbial lipases as well as an other source, such as, the lipase of leucocytes.

Table 10. Analysis of individual free fatty acids in unsalted and salted Cheddar cheese during ripening.

Lot A

Interval of testing	Butyric	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
					Unsalted *						
1 day	57.3	41.6	21.8	64.0	94.3	346.6	712.6	331.9	512.1	250.0	20.3
4 wk	64.4	66.7	35.6	111.5	181.9	653.7	992.4	552.1	763.7	158.0	24.2
8 wk	81.3	62.9	48.9	93.0	172.0	619.6	1338.3	541.1	831.8	150.0	25.9
12 wk	97.5	70.9	57.0	88.8	127.4	458.1	965.5	445.2	874.4	88.6	24.8
					Salted *						
1 day	43.3	43.2	27.8	70.0	109.9	387.2	448.0	434.3	423.1	267.7	38.9
4 wk	60.5	45.6	21.0	95.6	105.6	356.5	945.1	429.2	594.6	339.9	35.3
8 wk	61.5	63.0	32.3	124.1	117.1	391.4	988.1	460.9	594.3	396.5	70.6
12 wk	60.8	48.7	29.3	104.4	115.2	365.4	958.6	383.4	599.9	472.8	55.9

* mg acid/kg cheese

Continued

Table 10. (continued).

Lot B

Interval of testing	Butyric	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
					Unsalted						
1 day	32.3	28.3	19.5	37.4	61.4	198.3	547.5	228.5	422.6	60.5	8.6
4 wk	65.8	49.9	34.4	60.0	88.8	319.5	757.1	311.4	549.1	78.2	13.3
8 wk	124.1	89.0	70.4	150.1	196.0	848.1	1452.3	679.1	1202.8	99.5	46.8
12 wk	86.7	33.7	28.4	123.2	108.5	314.7	724.6	355.0	503.1	105.9	121.3
					Salted						
1 day	20.1	18.7	12.0	95.1	69.9	245.7	594.8	247.3	327.5	258.1	11.4
4 wk	49.4	34.3	23.3	81.6	99.9	334.6	806.1	428.9	480.1	328.7	26.0
8 wk	86.7	74.0	42.7	123.2	159.1	541.0	1241.0	500.2	944.2	113.2	17.8
12 wk	45.3	33.6	31.5	65.7	106.4	356.0	579.6	242.1	475.3	99.6	14.5

Continued

Table 10. (continued).

Lot C

Interval of testing	Butyric	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
					Unsalted						
1 day	8.3	42.2	29.0	67.4	76.7	261.5	638.4	378.4	994.7	227.5	37.1
4 wk	83.2	59.4	25.8	101.0	111.7	353.6	937.0	418.4	664.3	345.6	26.0
8 wk	41.3	50.7	17.6	161.8	260.4	828.3	1709.1	400.9	1596.4	362.6	55.8
12 wk	143.4	93.5	56.8	202.6	193.3	714.1	1780.9	827.1	1335.5	245.4	26.0
					Salted						
1 day	61.4	47.2	18.7	59.3	80.2	286.7	860.5	462.3	536.4	243.1	67.3
4 wk	134.5	86.1	58.4	144.5	184.7	708.6	1815.1	839.4	1607.4	178.0	20.5
8 wk	28.6	38.4	20.8	88.7	92.0	360.0	765.2	489.6	563.0	278.7	13.9
12 wk	35.2	27.1	12.1	71.7	92.8	310.7	614.8	294.7	415.7	369.5	23.9

Data are the average of duplicate tests.

CHAPTER V

SUMMARY AND CONCLUSION

Three lots of unsalted Cheddar cheese were made with salted controls and examined during a 12 wk ripening period. The following were the findings:

1. The flavor of unsalted cheese was flat, fermented, bitter and unnatural, and the body, weak, pasty and gassy during the first 12 wk of ripening. At the end of this period the flavor score for the unsalted cheese was 36.37-37.20 compared to 39.20-39.50 for the controls (salted) and the body score for the unsalted cheese was 26.87-27.30 compared to 29.0-29.37 for the controls. When 0.5-1.5% salt was incorporated into the ripened unsalted cheese, the flavor score was higher and at 1% salt closely compared with controls.

2. The shear force values for unsalted cheese were lower than those for the salted cheese. The ratio of shear force (salted/unsalted) varied from 1.8686 on the first day to 3.0425 at the end of 4 wk. The mean shear force value of 0.1302 $lb_{f/g}$ sample on the first day for the unsalted cheese steadily declined to 0.0890 $lb_{f/g}$ sample at 10 wk compared to the values 0.2433 $lb_{f/g}$ sample on the first day increasing to 0.3082 $lb_{f/g}$ sample at 1 wk and then steadily declined to 0.2503 $lb_{f/g}$ sample at 10 wk for the salted controls. A slight increase occurred at 12 wk in the shear force values of unsalted and salted cheese.

3. Average pH values for the unsalted cheese was 5.16 at 1 wk and increased steadily to a maximum of 5.29 at 7 wk. Then variations were observed. In all samples the pH of unsalted cheese remained lower than the controls, which had an average pH of 5.46 at 1 wk and 5.62 at 2 wk.

4. Soluble nitrogen expressed as the percentage of total nitrogen in the unsalted cheese samples was found to be between 35.96-41.11 compared to 21.39-29.49 in the controls at the end of 12 wk ripening.

5. The average volatile fatty acids in the unsalted cheese steadily increased from 22.90 ml to a maximum of 28.87 ml of N/10 acid per 100 g cheese during 1 wk. Increase in salted cheese was from 16.70 ml to 22.63 ml of N/10 acid per 100 g sample during 8 wk. During subsequent ripening the volatile fatty acids showed a variation in both salted and unsalted cheese, but remained higher in unsalted cheese.

6. Acetic acid was more in unsalted cheese during ripening despite the variations within the samples at different intervals. Mean values for acetic acid were 427.8 mg/kg at 1 day increasing to 515.5 mg/kg cheese at 10 wk for the unsalted and 285.7 mg/kg cheese at 1 day increasing to 386.1 mg/kg cheese at 8 wk for the salted controls. Acetic acid content increased in unsalted as well as the salted cheese at 14 wk ripening, after small decreases between 8-12 wk.

7. The salted and unsalted cheese samples had identical free fatty acids liberated in all trials. These were the even-numbered carbon fatty acids from butyric through linolenic. Based on the amounts of free fatty acids liberated during cheese-ripening, the extent of the fat hydrolysis in unsalted cheese was greater than in salted cheese. In most trials the free fatty acids increased initially and then decreased toward the end of the ripening period. Myristic, palmitic, stearic and oleic acids were present in higher amounts during the ripening of both kinds of cheese.

In conclusion, unsalted cheese ripened at a faster rate than the salted cheese. But, due to its less desirable flavor and the weak and

pasty body it has a lower acceptability. The manufacture of unsalted cheese with a better body and texture and flavor continues to be a challenge.

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