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Title: Time-Temperature Effects on Cheddar Cheese Ripening: an Interpretation of Microbial, Chemical and Sensory Changes.

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In spite of major research and process improvement efforts, there is still a wide variation in the sensory properties of the most popular cheese variety consumed, Cheddar cheese. Cooling of freshly formed cheese is believed to be a processing step requiring closer control to achieve uniform and consistent flavor quality.

A simple, rapid isocratic HPLC method was developed to quantify sugars and organic acids using a cation exchange column, and ultraviolet and refractive index detectors connected in series. Proteolysis extent, total acidity, pH, lactose and organic acids, as well as descriptive sensory evaluation by expert cheese judges were used to evaluate 60-day old Cheddar cheese from a major local processor. In a blind test, samples of acceptable and defective flavor quality were identified on the basis of statistically significant differences in organic acid profile and lactose level. Proteolysis extent was not a conclusive index, however, total acidity and pH appeared to follow the

quality difference identified by HPLC. This study suggests that objective measurements could complement subjective tests to facilitate the detection of flavor quality problems.

The effect of time and temperature on aging was studied using Cheddar cheese from the same processor. Small pieces cut from 18 kg blocks immediately after pressing were rapidly cooled to 12, 15, 20, and 25°C. Samples after various storage days were evaluated for proteolysis, total acidity, pH, sugar and organic acid profile. Proteolysis extent and concentration of most organic acids increased as a function of time and temperature. Lactose consumption and lactic acid production showed a non-significant temperature dependency. A kinetic analysis of organic acids production, changes in total acidity, lactose disappearance, and proteolysis extent generated parameters for theoretical and empirical equations for the effect of time and temperature on these indexes.

The experimental results obtained in this study as well as parallel studies on sensory and microbiological changes provide information on Cheddar cheese aging at constant and uniform temperature. This is not the case of a commercial cheese block, particularly during the initial cooling period. Therefore, a heat transfer model was needed to predict temperature as a function of time and location within the block. This information was combined with kinetic expressions for the effect of time and temperature on flavor quality to predict sensory and chemical characteristics at a large number ($>1,200$) of locations within the block. A computer optimization method was used in conjunction with the heat transfer and kinetic models to determine cooling and aging conditions that yield chemical and sensory indexes close to those obtained for commercial samples of desirable quality.

Time-Temperature Effects on Cheddar Cheese Ripening: an Interpretation of Microbial, Chemical and Sensory Changes

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Time-Temperature Effects on Cheddar Cheese Aging: an Interpretation of Microbial, Chemical and Sensory Changes

I. INTRODUCTION

USDA statistics indicate that cheese consumption in the United States is increasing at an annual rate of 8.5% and that Cheddar cheese is still the most popular variety consumed. The annual per capita consumption for Cheddar cheese is 9.8 lb, or 44% of the total cheese consumed (USDA, NASS 1990). A recent consumer preference test, surveying 2100 people, found that most respondents preferred the flavor of a 9 month old Cheddar cheese over that of a 6 month old Cheddar cheese (Webster and Frye, 1987). To save on costs of aging there is a need for greater understanding of the biochemical mechanisms responsible for the development of mature Cheddar cheese flavor (Peterson and Marshall, 1990).

Sources of desirable ripening enzymes in cheese include the milk itself, rennet, and starter and non-starter lactic acid bacteria. Through enzymatic degradation, the major cheese components, protein, carbohydrate, and fat, all contribute to the development of mature Cheddar cheese flavor. However, most of the carbohydrates left in cheese after pressing are converted to lactic acid within the first few days after manufacture, while decomposition of fat in Cheddar cheese is typically not extensive. On the other hand, during aging, 25 to 35% of the proteins present in Cheddar cheese are hydrolyzed to

peptides and amino acids (Peterson and Marshall, 1990).

Major research and process improvement efforts have been accomplished in the past to ensure uniform cheese quality (Kosikowsky and Mocquot, 1958; Kristoffersen, 1967; Law et al., 1979; Olson, 1980; Lawrence et al., 1984; Reinbold and Ernstrom, 1988; Grazier et al., 1991a,b). Unfortunately there is still a wide variation in the sensory properties of Cheddar cheese which has always been a problem. An early survey of cheese manufacturers showed that a lack of control in the cooling step from pressing to aging was responsible for considerable variation within lots (Vedamuthu et al., 1969). This seems to remain one of the factors explaining the lack of product uniformity. Several modes of cooling are used to lower the temperature of freshly formed Cheddar cheese. In some plants, freshly formed cheese (at 35 to 38°C) is pressed into 18 kilogram (40 lb) blocks and palletized as schematically illustrated in Figure I.1 (Yates, 1989). Palletized cheese is stored in cooling rooms with circulating air at 2 to 5°C. Cooling data obtained for this situation are shown in Figure I.2 where temperature profiles for three locations within the pallet are displayed. Large temperature differences could be observed between the center, halfway, and the outer layer of blocks on the pallet.

A different situation is observed in plants where cheese is formed into 290 kilogram (640 lb) blocks, placed first into a plastic bag, then into a wooden box, and finally cooled in an aging room at 2 to 5°C. As noted in a recent publication (Reinbold and Ernstrom, 1988) large differences exist between center and side temperatures which are even larger when the cooling temperature is lowered (Figure I.3).

The elevated temperature of the cheese in the cooling room promotes an undesirable, rapid metabolism of lactose, which can lead too often to atypically low pH and subsequent high acid (sour) and bitter off-flavors. This development points to the opportunity for achieving greater control of acid development in Cheddar cheese manufacture through improved temperature control of the cooling rate of the freshly formed cheese mass.

The main goal of current research in our laboratory is to suggest process changes to reduce the frequency of flavor quality problems through an improved cooling uniformity of Cheddar cheese blocks. On the basis of this goal the specific objectives corresponding to this research project were:

1. Development of a rapid, simple and specific method for the simultaneous determination of sugars and organic acids by High Performance Liquid Chromatography.
2. Evaluation of composition analysis as a means for potential detection of Cheddar cheese flavor quality problems.
3. Quantification and interpretation of the effect of heterogeneous temperature distribution on selected chemical indicator variables.

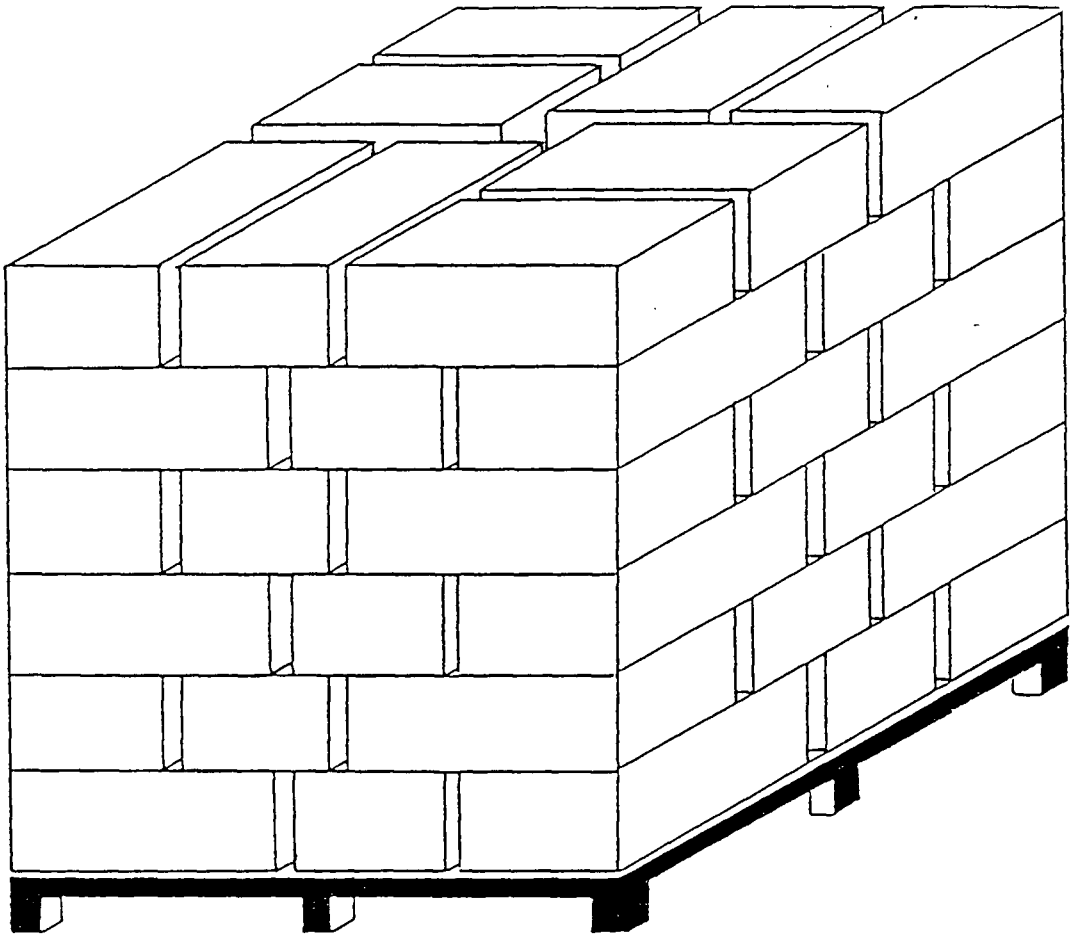

Figure I. 1 Arrangement of 18 kg (40 lb) Cheddar cheese blocks on a pallet

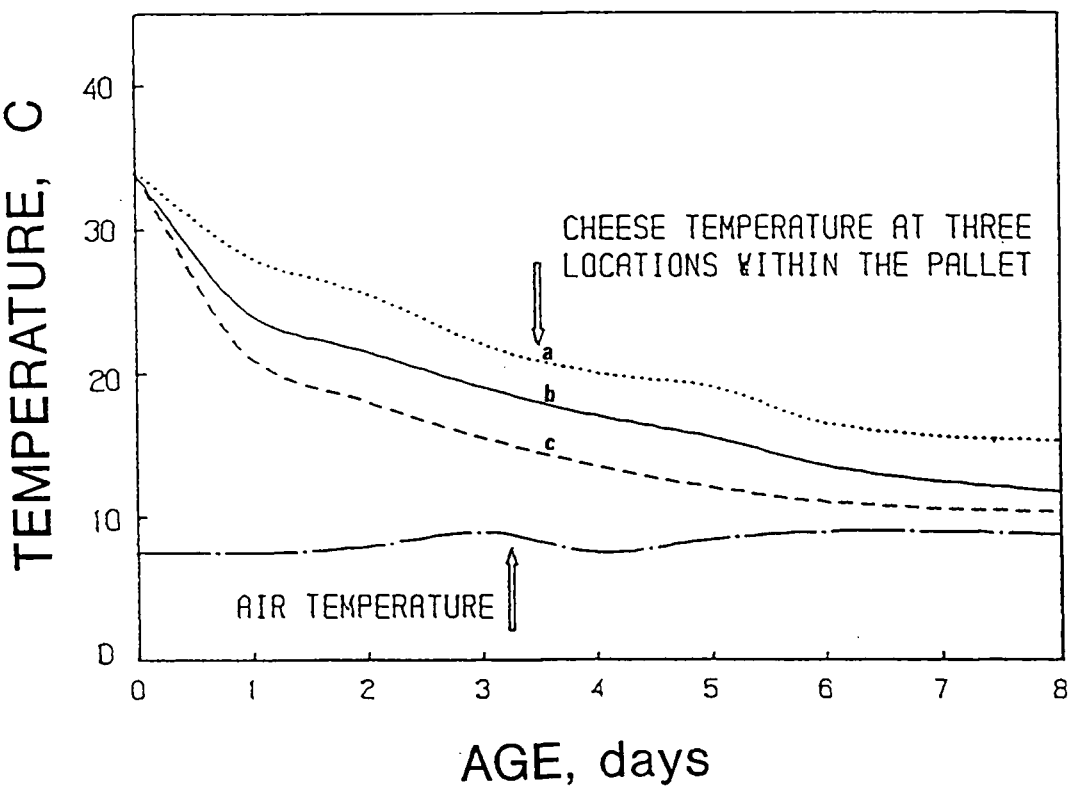

Figure I. 2 Change in temperature of palletized 18 kg (40 lb) Cheddar cheese blocks as a function of time in the cooling room (Yates, 1989):

a. Center of the pallet

b. Halfway within the pallet

c. Outer layer of the pallet

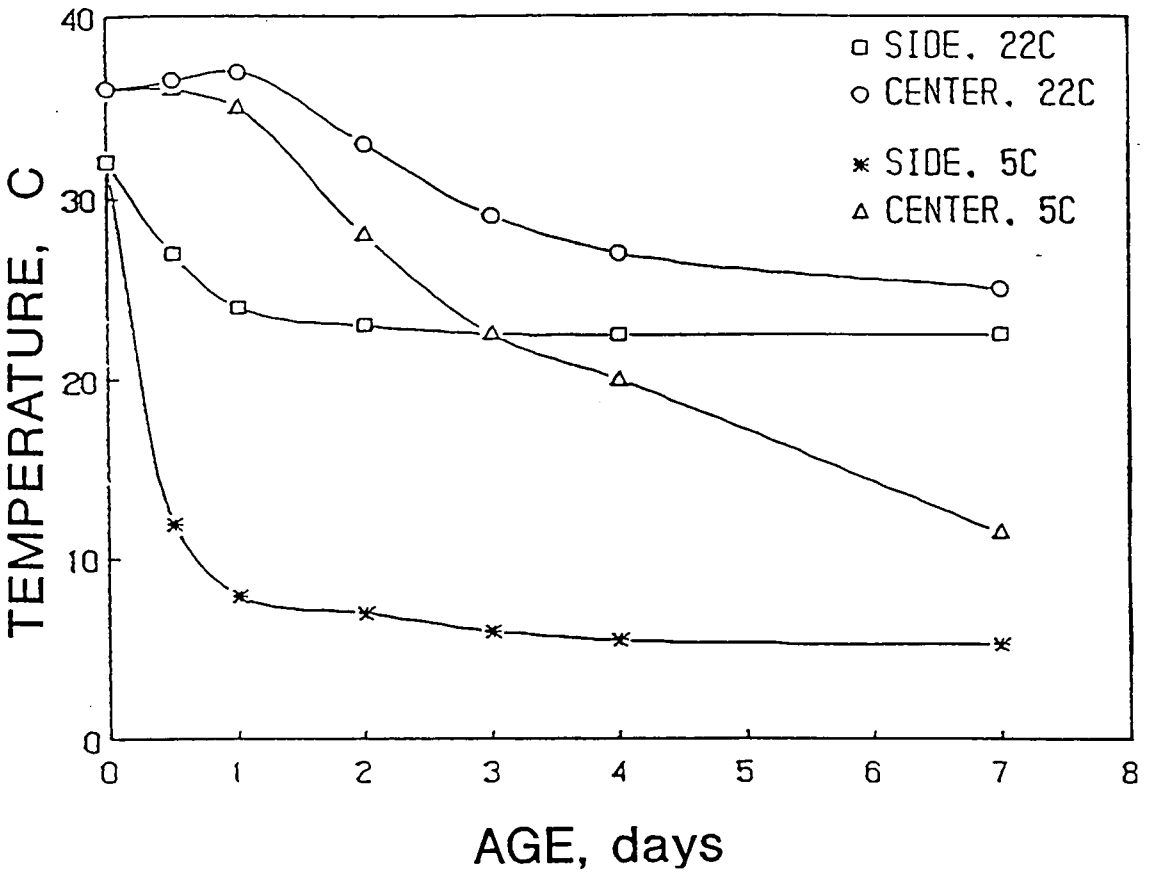

Figure I. 3 Temperature of 290 kg (640 lb) Cheddar cheese blocks as a function of storage time at two air temperatures (Reinbold and Ernstrom, 1988)

II. LITERATURE REVIEW

The origin of Cheddar cheese

The origin of cheesemaking is lost in antiquity. References both in the Bible and in the records of ancient Egypt indicate that the art of cheesemaking was well advanced by the time man began to record history. It is conceivable that nomadic tribesmen accidentally discovered the art while trying to store milk in animal stomachs. Over the centuries, the art has been refined and today, hundreds of cheese varieties are available (Moskowitz, 1980).

In the warm climates in which cheesemaking was first practiced, cheese would have tended to be of low pH as a result of the acid-producing activity of the lactic acid bacteria and coliforms in the raw milk. In colder climates, it would have been logical either to add warm water to the curds and whey to encourage acid production or to drain off the whey and pile the curd into heaps to prevent temperature falling. In the latter case, the piles became known as "cheddars" after the village in Sommerset, England, where the technique is said to have been first used about the middle of the 19th century. The concept of cheddaring was quickly adopted outside Britain. The first Cheddar cheese factory, as opposed to farmhouse cheesemaking, was in operation in the United States in 1851, followed by factories in Canada in 1864 and in New Zealand and England in 1871 (Lawrence and Gilles, 1987).

Originally, Cheddar cheese was apparently made by a stirred curd process without matting, but poor sanitary conditions led to many gassy cheeses with unclean flavors

(Kosikowski, 1977). Cheddaring was found to improve the quality of the cheese, presumably as a result of the greater extent of acid production. As pH fell below 5.4, the growth of undesirable, gas forming organisms such as coliforms is increasingly inhibited. The piling and repiling of blocks of warm curd in the cheese vat for about 2 hours tended to squeeze out of the cheese some pockets of gas formed during manufacture. Cheesemakers believed that the characteristic texture of Cheddar cheese was a direct result of the cheddaring process. It is now clear, however, that cheddaring and the development of the Cheddar texture are concurrent rather than interdependent processes (Lawrence and Gilles, 1987). The development of the fibrous structure in the curd of traditionally-made Cheddar does not commence until the curd has reached a pH of 5.8 or less. The changes that occur are a consequence of the development of acid in the curd and the loss of calcium and phosphate from the protein matrix (Lawrence et al., 1983; 1984).

Cheddar cheese manufacturing

Dramatic changes in the manufacture of Cheddar cheese have occurred during the past 20 years. The single most important factor has been the availability of reliable starter cultures. The development of continuous mechanized systems for cheese manufacture was constrained by the ability of the cheesemaker to control both the expulsion of moisture and the increase in acidity (Lawrence and Gilles, 1987). This in turn has led to the recognition that the quality of cheese, now being made on such a large scale in modern cheese plants, can only be possible if its chemical composition falls

within pre-determined ranges (Figure II.1) (Lawrence et al., 1984). Nevertheless, Cheddar cheese is still a difficult variety to manufacture since the long aging period necessary for the development of the required mature flavor can also be conducive to the formation of off-flavors.

Cheddar cheese texture, which is almost as important to consumers as its flavor, can vary considerably. The position of Cheddar cheese in the total cheese spectrum is particularly exemplified by its textural properties which lie between the crumbly nature of Cheshire and the plastic texture of Gouda (Figure II.2) (Lawrence et al. 1984).

The traditional manufacture of Cheddar cheese consists of: (a) coagulating milk containing starter culture with rennet; (b) cutting the resulting rennet coagulum into small cubes; (c) heating (scalding) and stirring the cubes with the concomitant production of a required amount of acid; (d) whey removal; (e) fusing the cubes of curd into slabs by cheddaring; (f) cutting (milling) the cheddared curd; (g) salting; (h) pressing; and, (i) packing; and (j) aging (Lawrence and Gilles, 1987).

To achieve uniform cheese in large commercial plants, manufacturing procedures must be as consistent as possible. The first requirement is uniform and good quality raw milk. This is achieved by bulking milk into a silo to even out differences in milk composition from the various farms supplying milk to the cheese plant. Fat should be standardized to a desired ratio of casein to fat. Under the same manufacturing conditions, the more fat in the milk for cheesemaking, and, therefore in the rennet coagulum, the more difficult it is to remove moisture because fat interferes mechanically with the process of syneresis (Sammis, 1910; Pearse and Mackinlay, 1989). The proportion of

rennet added should be the minimum necessary to give a firm coagulum in 30 to 40 minutes. The scalding temperature should be kept constant (38°C) throughout the entire cheesemaking season (Lawrence et al., 1984).

Given that the manufacturing variables are standardized, the most important factor by far in producing Cheddar cheese of uniform quality is the extent of acid production in the vats. To compensate for seasonal changes in milk composition it is normally only necessary to vary the percentage inoculum of starter added to achieve the required acidity at draining (Lawrence and Gilles, 1980).

Several aspects of the cheesemaking process influence the composition of the microflora in the finished product. Milk used for cheesemaking is usually heat-treated before the starter is added in an attempt to standardize the quality of the milk. The particular treatment used is determined by the manufacturer's preference for a balance in the type of bacteria and enzymes desired in the finished curd (Lau et al., 1990). The milk is then cooled to a temperature (approx. 26°C) adequate for growth of the starter inoculum. Acid production by the starter proceeds to the point at which coagulation by the rennet is optimal. This imparts a fresh acid flavor to the curd, assists in the formation of the rennet coagulum and, by causing shrinkage of the curd and moisture expulsion, promotes characteristic texture formation during cheesemaking (Chapman and Sharpe, 1981; Lawrence and Gilles, 1987). The temperature is then raised to approximately 30°C to facilitate the coagulation process.

Once the coagulum has formed to the desired firmness, the curd is cut, stirred, and scalded. Scalding is necessary to facilitate the shrinking of the protein matrix and

expelling of the whey from the curds, but care must be taken to avoid a temperature so high as to destroy the starter culture. Inhibition and destruction of a typical starter culture for Cheddar cheese begins at 40°C (Sandine, 1985).

As the curds and whey separate after cutting and scalding, they are piled and repiled to develop the characteristic texture of Cheddar cheese. This process tends to insulate the curd and retains a temperature in the upper 30°C range. Growth of the starter and concurrent acid production is therefore further promoted.

The curds are milled and salted to approximately 2%. This process is inhibitory to the starter bacteria and thus halts acid production at a pH in the range 5.2 to 5.3. At the time of loading into the hoops and pressing, the cheese temperature is approximately 30°C or higher (Westmark, 1991).

The aging process

Aging of Cheddar cheese is a process of digestion of different components by enzymes to induce chemical changes within the cheese, including proteolysis, glycolysis, and lipolysis (Chapman and Sharpe, 1981; Fox, 1989). During aging, the raw curd evolves from a rubbery elastic mass with an open coarse structure to a plasticized product with homogeneous texture with a close, smooth structure (Lawrence et al., 1987). The aging process may take months or even years depending on the degree of sharpness desired in the end product. The temperature of storage is generally between 4 and 15°C.

Two basic attributes of Cheddar cheese, flavor profile and physical structure, are determined mainly by chemical composition and the temperature at which the cheese is

aged (Figure II.3) (Lawrence and Gilles, 1980). It has been speculated that the rate of cooling of cheese after manufacture could be a factor affecting off-flavor development during aging (Fryer, 1982; Frederick et al., 1983). Aging is affected by the size of the block to the extent that there is non-uniformity of cooling throughout the block (Reinbold and Ernstrom, 1988).

Aging of Cheddar cheese occurs under anaerobic conditions. The rate of aging is controlled by the composition of the cheese, most especially the pH, moisture, salt level, and the temperature of the cheese. These factors all influence the microflora which develops in the cheese (Jensen et al., 1975; Chapman and Sharpe, 1981; Lawrence and Gilles, 1987; Reinbold and Ernstrom, 1988).

The microflora of heat treated milk used for cheese manufacture includes those organisms which have survived the heat treatment, and those which have contaminated the milk and/or cheese subsequent to the heat treatment process. This flora includes corynebacteria, micrococci, enterococci, sporeformers such as bacilli and clostridia species, staphylococci, coliforms, and lactic acid bacteria such as lactobacilli, pediococci, and leuconostoc. Added to this flora are the starter culture strains, usually *Lactococcus cremoris* (Reiter et al., 1967; Chapman and Sharpe, 1981; Cromie et al., 1987; Bhowmik and Marth, 1990).

During aging, only the non-starter lactic acid bacteria, including lactobacilli and pediococci, increase in numbers, while the others gradually die out (Reiter et al. 1967; Chapman and Sharpe, 1981; Thomas et al., 1985).

Lactococcus cremoris is the starter of choice for Cheddar cheese because it has

the advantages of producing good cheese flavors, being free from off-flavor production, and developing a steady rate of acid production from lactose (Chapman and Sharpe, 1981). This species is homolactic under the conditions of cheese manufacture, producing only small quantities of acetate and diacetyl from lactose (Thomas et al., 1979; Marshall and Law, 1984). Under conditions of low residual sugar availability, anaerobiosis, and controlled pH, several lactic lactococci strains have been found to revert to heterolactic fermentation patterns. Fermentation end products include mostly acetate and ethanol with as little as 1% conversion to lactate (Thomas et al., 1979).

Maximum levels of starter bacteria are reached at the milling stage, with *L. cremoris* reaching 10^8 CFU/gram. *L. cremoris* is inhibited by 2% salt, thus numbers begin to decline at salting (Chapman and Sharpe, 1981; Lawrence and Gilles, 1987). Starter bacteria die out during the aging process as do most other bacteria including enterococci and leuconostoc.

The dairy plant environment can become the permanent habitat of particular strains of adventitious microorganisms. Resident flora can infect each batch of cheese, such that a particular flora can impart individual flavor overtones to cheese made in that plant (Chapman and Sharpe, 1981). The concentration of NaCl in the cheese aqueous phase and the low pH are inhibitory to most spoilage bacteria. Most unwanted bacteria are suppressed by salt at the levels found in Cheddar cheese except for *Escherichia coli* which requires almost 12% NaCl for inhibition. Coliforms tolerate acid, salt, are not inhibited by starter bacteria, ferment lactose, and grow at temperatures found in cheesemaking. Their counts are kept low to prevent flavor and body defects in the early stages of aging.

Although non-starter bacteria do grow on sources other than lactose, the presence of lactose encourages their rapid growth. This tends to result in a more heterolactic metabolism of lactose, usually with the production of off-flavors. The initial numbers of non-starter bacteria in the salted curd can be controlled by attention to sanitation during manufacture. Thereafter, their rate of growth, particularly during the first days of aging, should be kept to a minimum, and this is controlled mainly by the cheese temperature and pH (Lawrence et al., 1984).

Lactobacilli reach counts of approximately 10^3 to 10^4 CFU/gram in the curd and counts of 10^6 to 10^8 after 10 to 60 days in the aging cheese (Grazier et al., 1991b). Since lactococci are faster growing organisms, the number of lactobacilli remain low until the greater acid tolerance of the lactobacilli allow these organisms to out-compete others. Lactobacilli metabolism is fermentative with at least half of the end product as lactic acid. Pyruvate, an intermediate in both homofermentative and heterofermentative pathways, may yield diacetyl and its derivatives, or acetic acid, ethanol and CO_2 (Kandler and Weiss, 1986).

Flavor development during Cheddar cheese aging

After more than 50 years of research, both the origin and the composition of Cheddar cheese flavor remain puzzling questions (Aston and Dulley, 1982; Vandeweghe and Reineccius, 1990). Kosikowski and Mocquot (1958) postulated the component balance theory for hard cheeses such as Cheddar cheese, i.e., flavor is not due to a single component but is the result of a synergistic blend of various compounds in proper

proportion. This theory has been accepted by researchers in spite of the disagreement on the relative contribution of the various components (Moskowitz, 1980; Aston and Dulley, 1982).

A number of flavor characteristics are associated with the microbiological flora of the cheese. Many of the components of Cheddar cheese flavor develop through enzymatic action. Starter and non-starter bacteria are sources of some of these enzymes (Moskowitz, 1980). Cheese made under asceptic conditions with glucono delta lactone instead of starter bacteria do not develop a typical Cheddar flavor (Reiter et al., 1967) indicating that the bacterial flora of the cheese are associated with flavor development. Although it has been postulated that starter lactococci may not be directly responsible for the development of characteristic flavor compounds, it has been demonstrated that they establish the proper chemical conditions of acidity and negative redox potential for production of flavor compounds associated with Cheddar cheese (Law et al., 1976; Law and Sharpe, 1978; Lawrence and Gilles, 1987). These authors postulate that bacterial enzymes released upon lysing of dead starter cells do not in themselves produce flavor components, except for a few low molecular weight precursors to aroma compounds, e.g., cysteine and methionine.

Non-starter lactic acid bacteria contribute to the production of flavor and aroma compounds in Cheddar cheese in three ways: 1) the production of trace aroma compounds, 2) the action of primarily intracellular proteases and lipases liberated during autolysis of dead cells, and 3) the conditions of low pH and negative redox potential which allow for the chemical production of reduced sulfur compounds as chief

constituents of Cheddar flavor (Chapman and Sharpe, 1981; Aston and Dulley, 1982).

Lactose fermentation by lactic acid bacteria constitutes the basis of glycolytic reactions during aging. Huffman and Kristoffersen (1984) suggested that the residual lactose in cheese is completely utilized by all bacteria present after no more than two months of aging. Conditions which favor the die-off of starter bacteria prior to complete utilization of lactose increase the risk of formation of off-flavor components through uncontrolled heterofermentative growth of non-starter bacteria (Fryer, 1982). Within a salt-in-moisture ratio of 4.6 to 6% the rate of metabolism of lactose is controlled by the temperature of the cheese during the first few days of aging, because this controls the rate of growth of non-starter bacteria such as lactobacilli and pediococci (Fryer, 1982; Lawrence et al., 1984). Fryer (1982) suggested that rapid cooling to 10°C limits the growth rate of heterofermentative non-starter organisms and thus their fermentation products. This suggests that initial lower temperatures, followed by higher temperatures could be a satisfactory storage program (Frederick et al., 1983).

The casein micelle may be considered as being a particle, spherical in shape, composed of a heterogeneous group of associated phosphoproteins. It consists of three principal components, α_{s1}-casein (55%), β-casein (25%), κ-casein (15%), and several minor components, notably, γ-casein (5%) (Brunner, 1976). The different caseins, are not evenly distributed throughout the micelle, particularly κ-casein which appears to be located mainly at the surface of the micelle. κ-casein could be divided into two distinct regions, namely the hydrophobic para-κ-casein and the hydrophilic glycomacropeptide.

Proteolysis is one of the essential roles of bacterial enzymes during aging and has

been found to be controlled largely through aging temperature (Aston et al., 1983; Lawrence et al., 1984; Fox, 1989; Cliffe and Law, 1991). Protein hydrolysis proceeds from insoluble protein to simple soluble compounds via enzyme action. The types of proteolytic enzymes present within the aging cheese are the rennet chymosin, milk plasmin, and bacterial proteases (Visser, 1981; Fox, 1989). Chymosin, an enzyme extracted from calf stomach, and added to milk, coagulates the milk by initially attacking κ-casein which destabilizes the casein micelles. Chymosin split the κ-casein at the junction of para-κ-casein and glycomacropeptide moieties. When this occurs, the macropeptide diffuses into the serum, its stabilizing influence is lost, and the micelles begin to coagulate (Dalgleish, 1987). During the aging process, residual chymosin continues to attack α_{s1}-casein and to a much lesser extent β-casein. The peptides produced are further degraded by endopeptidases produced by starter microorganisms. After cell death and lysis, starter peptidases continue to act on the partially degraded cheese protein (Cliffe and Law, 1991). These enzymes have optimum activity under neutral to alkaline pH conditions, but retain some activity under the acidic conditions found in aging cheese (Cliffe and Law, 1979).

Plasmin, the principal indigenous proteinase in milk, is thought to contribute to the aging of certain cheese varieties (Lawrence et al., 1983, 1987; Farkye and Fox, 1991). Plasmin activity in cheese can be distinguished fairly easily from that of calf rennet because plasmin specifically breaks down β-casein to γ-casein and proteose peptones. Plasmin is present in Cheddar cheese (Lawrence et al., 1983; Farkye and Fox, 1990) where it was thought to contribute little to ripening because the pH of Cheddar

cheese is unfavorable for its activity (Grappin et al., 1985). However, Noomen (1978) reported plasmin activity in Meshanger-type cheese (pH 5.1) after six weeks of aging at 13°C. Proteolysis of β-casein, the casein most susceptible to plasmin activity, has been observed in Emmental (Lawrence et al., 1983), Gouda (Visser and de Groot-Mostert, 1977), and Cheddar (Creamer, 1975) cheese indicating that plasmin activity in Cheddar cheese may have been underestimated as recently suggested by Farkye and Fox (1991).

In Cheddar cheese, 25 to 35% of the protein is made soluble with a high proportion of the breakdown products as peptides and amino acids (Chapman and Sharpe, 1981). Reiter et al. (1969) first demonstrated that the amino acid content of Cheddar cheese increased significantly due to proteolytic activity of lactic acid bacteria. O'Keefe et al. (1976) suggested that non-starter bacteria contribute to a greater diversity of small peptides and free amino acids. More recently, Lee et al. (1990b) demonstrated that homofermentative lactobacilli strains, when combined with starter cultures, contributed significantly to the rate of proteolysis. The liberated amino acids and peptides build up in the cheese to produce background flavors, while some amino acids, e.g. methionine, undergo reactions to form flavor molecules such as methanethiol (Law, 1984; Fox, 1989; Cliffe and Law, 1991). Amino acids may be reduced by microorganism to ammonia and organic acids or oxidized to CO_2 and amines (Dulley and Grieve, 1974).

Small chain peptides derived from the cleavage of α_{s1}-casein tend to be strongly hydrophobic. The formation of these peptides account for the bitter taste that develops during the aging of cheese (Lindsay, 1985; Lowrie, 1977). Lowrie and Lawrence (1972) suggested that rennet in Cheddar cheese would not contribute directly to the formation of

bitter peptides but provide predominantly non-bitter peptides of high molecular weight. Lemieux et al. (1989) suggested that the formation of bitter-taste components in cheese and cultured dairy products was influenced considerably by the strain of the starter culture. Manning (1978) found that bitterness reached a maximum intensity at 2 to 4 months when aged at 13 to 14°C, then declined rapidly to a minimum intensity at 10 months.

Lipolysis during cheese aging is primarily the result of milkfat hydrolysis catalyzed by various bacterial esterolytic and lipolytic enzymes. Lipases added to milk with the coagulating enzyme at the time of cheesemaking may contribute also to lipolysis in the cheese (Marth, 1963; Kamaly and Marth, 1989). Peterson et al. (1948) reported that the presence of lipase activity in extracts prepared from Cheddar cheese could catalyze the hydrolysis of tributyrin. Peterson and Johnson (1949) suggested that lactic acid bacteria has an intracellular lipase active between pH 5 and 6, which is liberated by bacterial autolysis and is able to attack milkfat. Singh et al. (1973) studied the extracellular and intracellular lipases of lactic lactococci. They found that intracellular lipase from *Lactococcus lactis* hydrolyzed tributyrin, but hydrolysis of tripalmitin and triolein was very limited. *L. cremoris* did not hydrolyze tripalmitin and triolein. El Soda et al. (1986) reported that several lactobacillus species possessed more lipolytic activity toward triglycerides with smaller rather than larger numbers of carbon atoms or milkfat emulsions. They found that the optimum rate of hydrolysis for both tributyrin and milkfat emulsions occurred near a neutral pH at 40 to 50°C.

Volatile fatty acids are produced in varying amounts in Cheese and have been

implicated as important flavor components (Dulley and Grieve, 1974; Lamparsky and Klimes, 1981). Fat hydrolysis products of great importance are the volatile lower fatty acids, especially butyric, caproic, caprylic, and capric. Nakae and Elliot (1965) showed that volatile fatty acids from acetic to caproic were produced from casein hydrolysates by all lactic lactococci strains examined. However, Dulley and Grieve (1974) reported that volatile fatty acids increased at a much lower rate in skimmilk cheese.

Effect of non-uniform cooling on cheese aging

Several studies have been conducted on the effect of aging temperature on the flavor quality of Cheddar cheese. Law et al. (1979) showed that the effect on flavor intensity of aging cheese at a low (6°C) and a traditional (13°C) aging temperature was greater than either the presence of non-starter lactic acid bacteria or the type of starter culture used. Reinbold and Ernstrom (1988) suggested that the most desirable situation would be uniform and rapid cooling of all positions within a cheese block but this is quite difficult to achieve, particularly in large blocks.

Researchers have found the rate of cooling of the cheese in the first days of aging to be the most important factor in controlling cheese flora, particularly non-starter bacteria (Fryer, 1982). Research has shown widely varying growth rates of non-starter bacteria at different constant cooling/aging temperatures (Grazier et al., 1991b). Miah et al. (1974) suggested that proper cooling temperature offers an effective approach to control the development of cheese flavor.

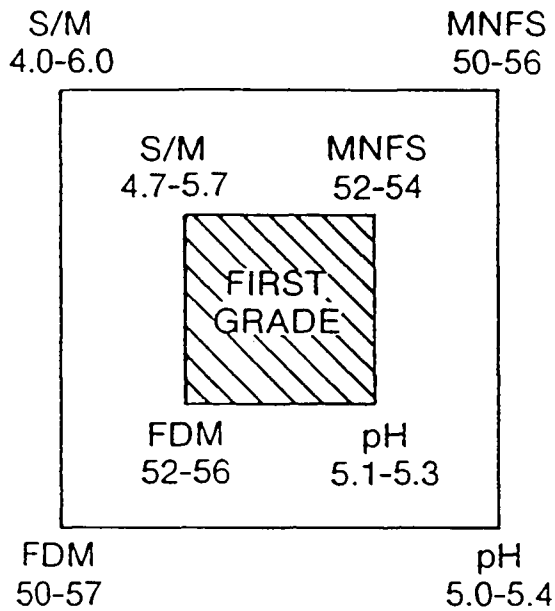

Figure II. 1 Suggested ranges for salt-in-moisture (S/M), moisture in non-fat substance (MNFS), fat-in-the-dry matter (FDM), and pH for First grade (shaded) and Second grade Cheddar cheese (Lawrence and Gilles, 1987)

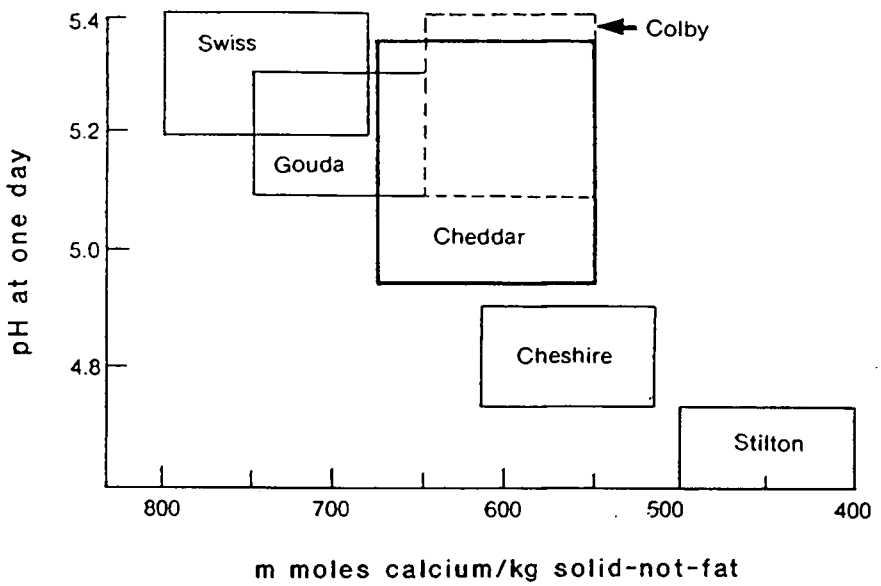

Figure II. 2 Classification of traditionally manufactured cheese varieties by their characteristic ranges of calcium/solids-non-fat and pH (Lawrence and Gilles, 1987)

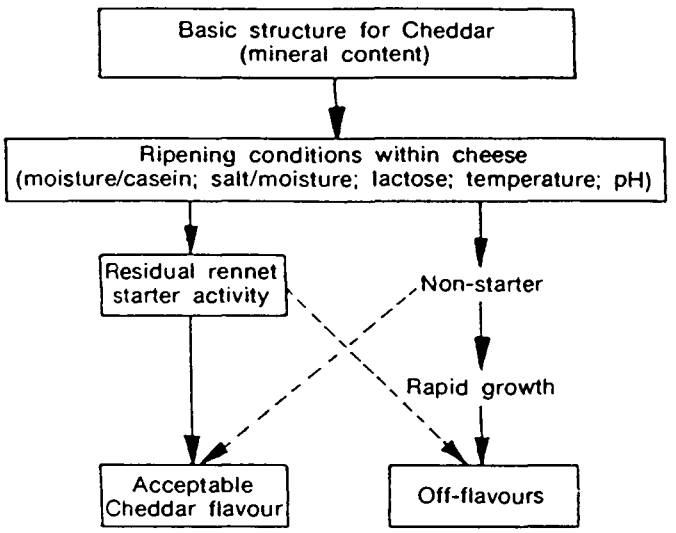

Figure II. 3 The main factors that determine the development of flavor in Cheddar cheese (Lawrence and Gilles, 1987)

III. SIMULTANEOUS DETERMINATION OF SUGARS AND ORGANIC ACIDS IN CHEDDAR CHEESE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

ABSTRACT

In a simple, rapid isocratic HPLC method sugars and organic acids were separated on an Aminex HPX-87 column in the H^+ form and detected using ultraviolet and refractive index detectors in series. Sugars (lactose, glucose and galactose) and acids (orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric and hippuric) were identified by retention times. This method affords a simple technique for monitoring starter culture activity and following quality changes during cheese aging.

INTRODUCTION

Quantitative determination of sugars and organic acids in dairy products could be used to monitor starter culture activity, understand microbial metabolism and follow quality changes during cheese aging. American Cheddar is the main cheese type produced in the United States (USDA NASS, 1989). Major research and process improvement efforts have been accomplished to ensure uniform cheese quality (Kosikowski and Mocquot, 1958; Kristoffersen, 1967; Law et al., 1979; Olson, 1980; Lawrence et al., 1984; Reinbold and Ernstrom, 1988; Grazier et al., 1990). Unfortunately, there is still a wide variation in the sensory properties of Cheddar cheese, which is somewhat traditional and assumed. Experienced cheese graders have been reported to critique 30-40% of all American Cheddar cheese as "high acid (sour) and/or bitter (aged cheese)" (Bodyfelt, 1986; Bodyfelt et al., 1988).

An important factor in producing cheese of uniform flavor quality is the extent of lactose utilization for acid production in the vat stage. Another is the subsequent microbial activity of residual starter culture and non-starter bacteria during cheese aging (Gilles and Lawrence, 1973; Lawrence et al., 1983; Lawrence et al., 1984). Numerous analytical methods have been employed to analyze organic acids or sugars in dairy products (Marsili et al., 1981; Pirisino, 1983; Woolard, 1983; Huffman and Kristoffersen, 1984; Bevilacqua and Califano, 1989). However, no attempt has been reported on simultaneous determination of those components. The objective of our research was development of a rapid, simple and specific method for simultaneous determination of sugars and organic acids in Cheddar cheese by HPLC.

MATERIALS AND METHODS

Apparatus and operating conditions.

The Shimadzu liquid chromatography system (Shimadzu Scientific Instruments, Columbia, MD) consisted of a LC-6A solvent delivery unit, a RID-6A refractive index detector, a SPD-6AV variable wavelength UV/Visible detector, a CTO-6A column oven and a CR501 Chromatopac data processor. The detectors were connected in series. The UV detector, set at 220 or 285 nm, was used for quantification of organic acids. The RI detector was used for quantification of sugars. The analysis was performed isocratically at 0.7 mL/min and 65 °C. We used a 300 mm x 7.8 mm i.d. cation exchange column (Aminex HPX-87H) with a Cation H^+ Microguard cartridge (Bio-Rad Laboratories, Richmond, CA). Mobile phase was 0.009N H_2SO_4 filtered through a 0.45 μm membrane filter (Nuclepore Corp., Pleasanton, CA) and degassed by sonication under vacuum.

Calibration and calculations

Single standard solutions of sugars and acids were prepared to establish elution times. Quantification was based on the external standard method. Mixed standards for sugars (glucose, galactose and lactose; Sigma Chemical Co., St. Louis, MO) and organic acids (citric, orotic, pyruvic, lactic, uric, formic, acetic, propionic, butyric and hippuric; Sigma Chemical Co.) were prepared to establish calibration curves. Resulting peak heights were determined for duplicate 20 μL injections. Best fit standard curves for each component were prepared by linear regression of peak height vs. concentration. Afterwards, individual standard solutions were added one by one to Cheddar cheese

samples to verify correct identification of peaks using elution times.

Citric and orotic acids coelute under chromatographic conditions listed. Analyzing samples at two different wavelengths enabled quantification of citric and orotic acids; the latter was determined at 285 nm where citric acid does not absorb. Lactose and citric acid coeluted in a non-additive manner. To solve this problem, fifteen solutions containing known concentration ratios of citric acid and lactose were prepared within the range expected. They were chromatographed in duplicate using both detectors (UV and RI). Multiple regression analysis was then applied using lactose concentration as dependent variable and citric acid concentration (UV) and RI total peak area as independent variables. In this case peak area gave better correlation ($r = 0.9996$) than peak height, and hence the peak area was used to reflect concentration. The following multiple regression equation was used to obtain lactose concentration in Cheddar cheese samples:

Lactose (ppm) = 36.5 + 0.0027 (total RI peak area) - 1.27 (citric acid UV, ppm)

Sample preparation

Cheddar cheese samples were withdrawn from the summer production (June 1989) of a commercial cheese manufacturer (Tillamook Creamery Assn., Tillamook, OR). Cheddar cheese blocks (18 kg) were cut into pieces directly after the pressing operation; they measured 6.4 x 8.9 x 14.6 cm. Each piece was vacuum shrink-wrapped in commercial O_2-barrier cheese film. Samples were placed in an incubator set at 12°C.

After the desired storage time, samples were extracted using a modification of the method of Bevilacqua and Califano (1989). Twenty-five mL of 0.009N H_2SO_4 (mobile phase) was added to 5 g of ground Cheddar cheese and extracted for 1 hr while mixing with a magnetic stirrer. The extract was centrifuged at 5,000xg for 10 min. The supernatant was filtered through Whatman #1 filter paper and through a 0.20 μm membrane filter (Bio-Rad Laboratories, Richmond, CA). Duplicate analyses were performed for all samples.

Recovery studies

The efficiency of the extraction procedure was evaluated by addition of organic acids and sugars to Cheddar cheese samples. A known amount of standard solution of sugars or organic acids was added to 5 g of cheese and extracted as described above. The amounts added were roughly 50% of the true concentration of the samples. Most components yielded high percent recoveries with exception of citric and hippuric acids (Table III.1). The low citric acid recovery was surprising because of its high solubility in the extraction solution.

RESULTS AND DISCUSSION

Simultaneous determination of sugars and organic acids in Cheddar cheese samples was made possible by using the cation-exchange column in the hydrogen form with the UV and RI detectors connected in series. Typical chromatograms depicting separation

of aqueous standard solutions of sugars and organic acids are shown in Figure III.1. Detector response, was linear for all acids and sugars; correlation coefficients indicated linear responses (Table III.1). Efficiency of the extraction procedure, measured by adding to a Cheddar cheese sample known amounts of standard solutions containing the acids and sugars ranged from 70-105% (Table III.1). Use of two different UV detector wavelengths was useful for qualitative identification and also quantification of orotic and citric acids in sample chromatograms.

Fresh Cheddar cheese samples stored at a constant temperature of 12°C for 0, 8, and 48 days as well as 60-day aged commercial cheese were analyzed. The organic acid and sugar composition results are presented in Table III.1. Lactose concentration decreased with storage time from an initial high level of 7.25 to 3.85 mg/g cheese (dry basis) after 8 days storage. No lactose was found in the 48 day samples nor the commercial cheese. Only residual galactose was detected which served to confirm, as generally acknowledged, that glucose was the preferred microbial substrate of the two lactose hydrolysis end products. The production of lactic acid continued during aging (storage); it increased from 19.5 mg/g cheese (dry basis) at time 0 to 28.3 after 48 days. The decrease in orotic acid concentration was in agreement with previous observations. Orotic acid has been reported to be readily utilized by various bacteria used in fermentation of dairy products (Chen and Larson, 1971; Empie and Melachouris, 1977; Larson and Hegarty, 1979). Also, propionic acid increased with storage time from 0.74 at day 0 to 1.82 mg/g cheese at day 48. The lower concentrations found for propionic, lactic and butyric acids in the commercial sample could possibly be attributed to

differences in storage temperatures. The effects of cooling rates on commercial Cheddar cheese quality are the subject of current investigations in our laboratory.

CONCLUSIONS

The described procedure seemed well suited for analysis of various sugars and organic acids in dairy products. The main advantages of this method are simple sample preparation (a single extraction), low costs of extraction solvent and mobile phase, and use of a single HPLC column. A potential useful application of this method may be the monitoring of starter culture activity and Cheddar cheese aging. Cheese manufacturers must decide which lots (vats) of cheese are better for immediate sale and which lots should be committed to additional storage time for aged (sharp) cheese. Our technique could also offer a simple solution for monitoring bacterial activity in other types of fermented foods.

Table III. 1 Sugar and organic acid composition (mg/g cheese, dry basis) of Cheddar cheese samples[a]

	Recovery %	Storage (days) 0	8	48	Commercial (>60)
Sugars[b]					
lactose	95	7.3 ± 0.2	3.9 ± 0.05	nd[c]	nd
glucose	103	nd	nd	nd	nd
galactose	104	nd	0.32 ± 0.02	0.20 ± 0.02	0.24 ± 0.002
Acids[b]					
citric	70	2.2 ± 0.01	2.3 ± 0.07	2.1 ± 0.10	2.2 ± 0.16
orotic	96	0.04 ± 0.001	0.03 ± 0.0005	0.02 ± 0.0005	0.02 ± 0.0003
pyruvic	91	0.036 ± 0.001	0.039 ± 0.005	0.041 ± 0.004	0.074 ± 0.001
lactic	100	19.5 ± 0.5	21.0 ± 0.04	28.3 ± 0.05	26.5 ± 0.08
uric	80	nd	nd	nd	nd
formic	105	nd	nd	0.3 ± 0.06	0.6 ± 0.10
acetic	98	nd	nd	0.40 ± 0.02	0.30 ± 0.006
propionic	100	0.74 ± 0.03	1.05 ± 0.05	1.82 ± 0.06	0.98 ± 0.01
butyric	100	nd	0.50 ± 0.03	0.52 ± 0.02	0.20 ± 0.003
hippuric	80	nd	nd	nd	0.006 ± 0.0001

(a) Uncertainties represent average deviations of complete duplicate determinations. Concentrations were not corrected by recovery factor.

(b) All correlations were >0.9980

(c) nd = not detected

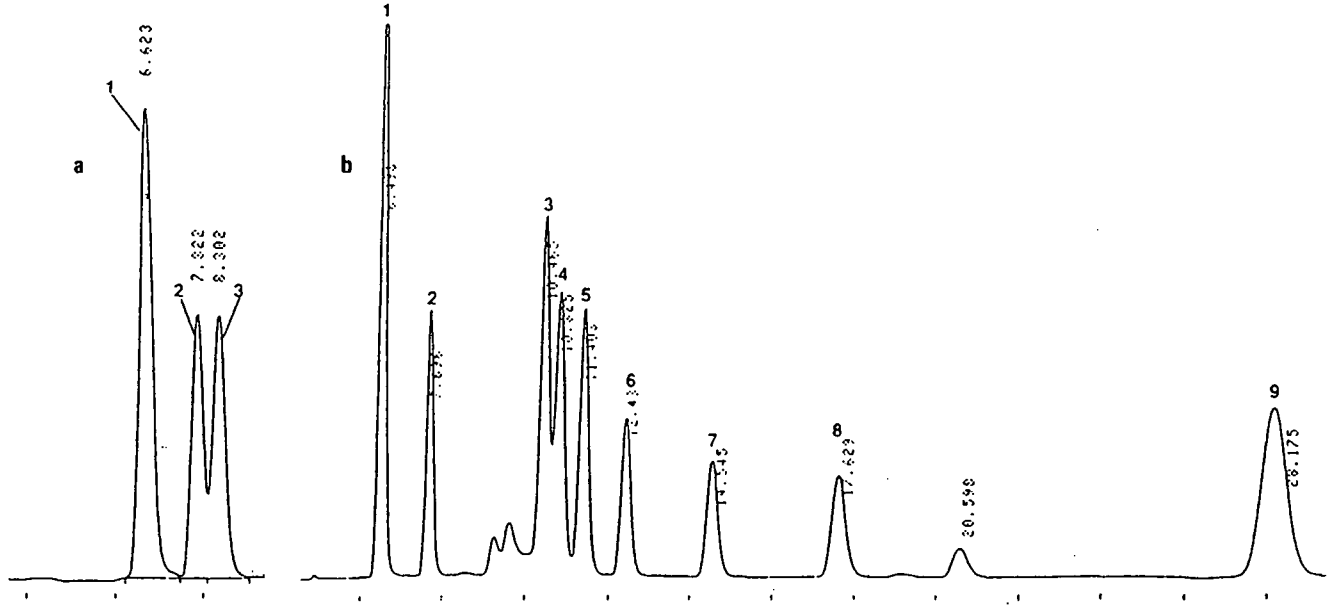

Figure III. 1 Typical Chromatogram of aqueous standard solutions.

(a) Sugars from RI detector.
Sugar standards: 1 = lactose, 2 = glucose, 3 = galactose.

(b) Organic acids from UV detector at 220 nm.
Acid standards: 1 = citric, 2 = pyruvic, 3 = lactic, 4 = uric, 5 = formic, 6 = acetic, 7 = propionic, 8 = butyric, 9 = hippuric

IV. OBSERVATIONS ON A POTENTIAL MEANS FOR OBJECTIVE ASSESSMENT OF CHEDDAR CHEESE FLAVOR DEFECTS

ABSTRACT

Proteolysis assessment, total acidity, pH, and determination of lactose and organic acids by HPLC, as well as descriptive sensory evaluation by expert cheese judges were used to evaluate 60-day old commercial Cheddar cheese from a major local dairy processor. In a blind test, samples of acceptable and defective Cheddar cheese quality were identified on the basis of statistically differences in organic acid profile and lactose level. Proteolysis extent was not a conclusive index, however, total acidity and pH appeared to follow the quality difference identified by HPLC. This study suggests that objective measurements could complement subjective tests and thus facilitate the detection of Cheddar cheese flavor quality problems.

INTRODUCTION

American Cheddar is the major type of cheese produced in the United States (USDA NASS, 1990). Major research and process improvement efforts have been accomplished in the past to ensure uniform cheese quality (Kosikowski and Mocquot, 1958; Kristoffersen, 1967; Law et al., 1979; Olson, 1980; Lawrence et al., 1984; Reinbold and Ernstrom, 1988; Grazier et al., 1990, 1991a,b). Unfortunately, there is still wide variation in the sensory properties of Cheddar cheese. Experienced U.S. cheese graders critique the flavor characteristics of Cheddar cheese to the extent that perhaps as much as 30-40% of it is "high acid (sour) and/or bitter" for medium and aged cheese (Bodyfelt, 1986; Bodyfelt et al., 1988).

An important factor in producing cheese of uniform flavor quality is the extent of lactose utilization for acid production in the vat stage, and also the subsequent microbial activity of residual starter culture and non-starter bacteria during cheese aging (Gilles and Lawrence, 1973; Lawrence et al., 1983; Lawrence et al., 1984). Rapid cooling of cheese blocks to aging temperature is the primary means of control of this microflora activity and promotes homofermentative metabolism (Fryer, 1982).

A survey of Cheddar cheese manufacturers showed that a lack of control in the cooling step from pressing to curing was responsible for considerable variation within lots (Vedamuthu et al., 1969). Cheese flavor quality is conventionally graded at day 60 for the purpose of determining the most suitable market for the cheese according to its potential for further aging (maturation). Storage of cheese for aging accounts for a

substantial part of the cost of making cheese since maintaining large storage areas at low temperature is expensive. Therefore, assessment of potential quality is of great economic importance to commercial cheese manufacturers (Manning et al., 1984).

Subjective procedures currently used to assess cheese quality at an early stage are not a reliable guide to consumer acceptability after cheese aging (Lawrence and Gilles, 1980). Assessment of cheese quality, or potential quality, using objective or instrumental methods of analysis would provide information not available to graders and thereby complement their knowledge of the cheese obtained by sensory methods (Manning et al., 1984). Composition analysis can help provide an objective method for detecting atypical cheese (Lawrence and Gilles, 1987).

The main purpose of this study was the evaluation of a potential detection of Cheddar cheese flavor quality problems using composition analysis. HPLC determination of sugars and organic acids, proteolysis extent, pH and total acidity were the characteristics chosen.

MATERIALS AND METHODS

Reagents

Sugars (glucose, galactose, lactose), organic acids (citric, orotic, pyruvic, lactic, formic, acetic, propionic, and butyric), glycine, sodium tetraborate, and trinitrobenzenesulfonic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Other analytical reagents were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ).

Cheddar cheese samples

In August 1990, a commercial processor (Tillamook County Creamery Assn., Tillamook, OR) selected Cheddar cheese with and without flavor defects using three in-house graders from different production lots. Cheese produced at this plant is made from heat-treated milk (65°C, 35s). Samples (ca. 250 g) obtained from 18 kg (40 lb) blocks aged for 60 days at 4°C were subjected to blind-random sensory and chemical tests. Sample numbers (1-8) were assigned after completion of data analysis.

Sensory evaluation

Single samples, tempered to 10-12°C before serving, were sensorially evaluated in blind tests by an expert cheese judge following USDA guidelines (Bodyfelt et al., 1988). A cheese core trier was used to obtain samples free of surface oxidation artifacts.

HPLC analysis

Sugars and organic acids were evaluated following a modification of the procedure described by Bouzas et al. (1991a). The Shimadzu liquid chromatography system used (Shimadzu Scientific Instruments, Columbia, MD) consisted of a LC-6A solvent delivery unit, a RID-6A refractive index (RI) detector, a SPD-6AV variable wavelength UV/Visible detector, a CTO-6A column oven and a CR501 Chromatopac data processor. Under the reported chromatographic conditions, lactose and citric acid coeluted in a non-additive manner when peak heights were used. Peak areas were found to be additive and were therefore preferred in this case. Citric acid RI peak area was estimated using UV

data and citric acid calibration response data for the RI detector. Lactose signal was then determined by subtracting calculated citric acid area from total peak area.

Sample preparation. Approximately 30 g of Cheddar cheese were ground in a Braun Aromatic Coffee grinder (Braun Inc., Lynnfield, MA) and 5 g extracted with twenty five mL of 0.009N H_2SO_4 (mobile phase) as described by Bouzas et al. (1991a). Duplicate analyses were performed for all samples.

Proteolysis evaluation

Proteolysis extent was evaluated by measuring free amino groups using trinitrobenzenesulphonic acid (TNBS) (Kuchroo et al., 1983; Polychroniadou, 1988). Duplicate 1 g cheese samples were dispersed in 20 mL borate buffer (0.1M $Na_2B_4O_7$, pH 9.5), warmed at 45°C for 15 min with stirring, and then centrifuged at 3,000xg for 20 min. A 3 mL aliquot of the supernatant was diluted with distilled water to 100 mL. A 0.5 mL portion of this extract was added to 0.5 mL borate buffer. Next, one mL TNBS reagent (1 mg/mL) was added. After thorough mixing, the solution was incubated at 37°C for 60 min. Blanks were prepared with 0.5 mL of H_2O instead of cheese extract. The reaction was stopped by adding 2 mL of 0.1M NaH_2PO_4 containing 1.5 mM Na_2SO_3 and the absorbance was measured at 420 nm. Glycine solutions (0.054 to 0.54 mM) were used as standards. A linear relationship was obtained between glycine concentration and color yield (r^2 = 0.9999).

pH and total acidity

A microprocessor pH/millivolt meter (Model 811; Orion Research Inc., Cambridge MA) with a combination spear-tip electrode (Model 91-63; Orion Research Inc.) was used for pH measurements. An X-shaped hole was cut into cheese samples and the electrode was inserted to a depth of 18 mm. Measurements were conducted in triplicate.

Total acidity was determined using the AOAC official method (AOAC, Official Method 16.276, 1984) and the results were expressed as g lactic acid/100 g cheese.

Statistical analysis

Data were analyzed through analysis of variance techniques using the General Linear Model on the SAS statistical software package (Version 6.02, SAS Inst. Inc., Cary, NC). Least significant differences (LSD) were used to determine statistical differences between the means ($P < 0.05$) for samples (1-8) and groups (acceptable-defective).

RESULTS AND DISCUSSION

Sensory evaluation

The flavor evaluations by the expert cheese judge and those by three processor's in-house graders were in close agreement. Three out of the eight samples tested (#1-3) were graded as typical, young Cheddar cheese. Samples 4 and 5 were also reported as young Cheddar cheese, but with a slight or moderate acid (sour) taste. The overall flavor characteristic for these five cheese samples was depicted as clean, moderately aromatic

and pleasantly acidic. For all five samples, cheese body and texture was described as being of exceptionally good quality. The cheese samples all possessed desired smoothness, meatiness, waxiness, and silkiness and were entirely free from gas holes. Cheddar cheese with all the aforementioned flavor, body and texture characteristics could be readily marketed as good quality mild Cheddar.

On the other hand, flavor defects were noted in samples 6 to 8. Sample 6 was described as practically devoid of flavor (flat), slightly sweet, and being a "curdy-like, 2-week old cheese". These sensory defects can be associated with an early die-off of the starter culture. Sample 7 presented a flat (lacking) acid flavor when initially tasted. This particular flavor defect is not usually considered serious or particularly objectionable for a young Cheddar cheese, since full cheese flavor may eventually develop with additional aging time. The evaluation of sample 8 suggested evidence of delayed lactose utilization and an abnormal fermentation. It was described as having a "whey-taint" off-flavor.

Objective evaluation

Lactose and organic acids content, proteolysis extent, total acidity, and pH were selected as quality indicator variables (Table IV.1). Glucose and galactose were not detected in commercial cheese samples (< 10 mg/100 g cheese). The levels of organic acids found in this study represent amounts well above typical flavor thresholds (0.03-10 mg/100 g cheese), and are expected to contribute to the overall sensory quality of Cheddar cheese.

For statistical analysis purposes and based on the sensory evaluation tests, samples

were assigned to one of two quality groups: "acceptable" (samples 1 to 5) and "defective" sensory quality (samples 6 to 8). Analysis of variance was then conducted by group. All indicator variables showed statistically significant differences ($P < 0.05$) between groups, except for proteolysis which showed no significant difference.

Analysis of variance was conducted also by individual sample. Statistically significant differences (LSD) for each indicator variable are shown in Table IV.1. Samples 1 to 5 showed similar organic acid profiles, as well as a comparable residual lactose content, and similar total acidity and pH value. Sensory evaluation portrayed a balanced and desirable flavor profile. Therefore, these chemical indicators could be regarded as characteristics of a balanced composition, and partially contributing to a desirable Cheddar cheese flavor. Samples 4 and 5, reported to have a slightly acid (sour) character had higher propionic acid concentration, and higher but not statistically significantly different (significance level of 5%) total acidity and acetic acid levels.

Samples 6 to 8 differed in organic acid profile, lactose content and total acidity from samples 1 to 5 (Table IV.1). Sample 8 showed low concentrations of citric acid, high concentrations of acetic and pyruvic acids, low levels of lactic acid and quite high levels of lactose. The high lactose content probably corroborated sensory evidence of high amounts of whey retained in the cheese.

Production of cheese relies mainly on starter activity to remove lactose (Lawrence et al., 1984). Starter bacteria must survive in sufficient numbers after the salting step to provide a desired final pH after one day (Lawrence and Gilles, 1980). Sample 6 could represent a case in which the starter bacteria did not survive in sufficient numbers.

Unusually high residual lactose levels along with low lactic acid concentration support our suggestion (Table IV.1). Lipolytic activity was not very evident as indicated by the low concentration of butyric acid. Lower total acidity and higher pH values clearly reflected the levels of organic acids and lactose discussed above.

Sample 7, classified by the expert judge as acid-flat and somewhat crumbly in body, had noticeably higher lactic acid concentrations and less residual lactose levels than samples 6 and 8. However, lactose levels were much higher than those expected for 60-day old Cheddar cheese.

Proteolysis data were not conclusive. No statistically significant differences were found among the eight samples. However, isovaleric acid, a metabolic product derived from the amino acid valine, showed statistically significant differences between both cheese groupings.

CONCLUSIONS

The use of selected objective indicator variables could be a useful monitoring strategy in the early detection of Cheddar cheese flavor defects. Assessment of cheese quality, or potential quality, by sensory evaluation could be efficiently complemented and reinforced with objective analytical data of the type presented here. The sensitivity of the objective measurements could be further enhanced by examining various parametric ratios, i.e. lactose/lactic, lactose/citric, lactose/total acidity.

Table IV. 1 Objective indicator variables for the potential detection of Cheddar cheese flavor defects [1,2]

	ACCEPTABLE QUALITY[3]					DEFECTIVE QUALITY[3]		
SAMPLE #	1	2	3	4	5	6	7	8
Lactose[4]	52^{a}	13^{a}	20^{a}	45^{a}	23^{a}	1221^{b}	556^{c}	1133^{d}
Acids [4]								
Citric	188^{a}	192^{a}	203^{a}	181^{a}	199^{a}	141^{c}	243^{c}	94^{d}
Pyruvic	1.2^{a}	1.1^{a}	1.2^{a}	1.6^{a}	1.1^{a}	3.7^{b}	1.0^{a}	6.8^{d}
Lactic	2900^{c}	3450^{a}	3300^{b}	3490^{a}	3310^{b}	1083^{d}	2297^{e}	1179^{f}
Acetic	22^{a}	18^{a}	19^{a}	35^{a}	21^{a}	30^{a}	38^{a}	73^{b}
Propionic	23^{a}	21^{a}	23^{a}	32^{bd}	38^{bc}	27^{ad}	27^{ad}	41^{c}
Butyric	15^{b}	10^{a}	12^{a}	11^{a}	18^{c}	4^{d}	6^{e}	5^{de}
Orotic	2.9^{a}	2.2^{b}	3.0^{a}	1.9^{b}	2.7^{a}	6.5^{c}	0.5^{d}	6.3^{c}
Isovaleric	46^{ad}	38^{bc}	48^{a}	57^{e}	41^{cd}	33^{b}	54^{e}	41^{cd}
Proteolysis[5]	125^{a}	114^{b}	127^{ac}	133^{c}	124^{a}	122^{a}	126^{a}	133^{c}
Total Acidity[6]	1.35^{ab}	1.45^{bc}	1.47^{bc}	1.57^{bc}	1.58^{c}	0.63^{d}	1.25^{a}	0.82^{e}
pH	5.00^{a}	5.06^{a}	5.00^{a}	5.01^{a}	5.03^{a}	5.76^{b}	5.02^{a}	5.70^{b}

(1) Average of two separate determinations from the same cheese sample
(2) Means with the same superscript within the same row are not significantly different from each other ($P < 0.05$)
(3) flavor quality assessed by expert cheese judge
(4) mg/100 g cheese, dry basis
(5) mmoles glycine/100 g cheese, dry basis
(6) g lactic acid/100 g cheese, dry basis

V. CHARACTERIZATION AND INTERPRETATION OF TIME-TEMPERATURE EFFECTS ON CHEMICAL CHANGES OCCURRING DURING CHEDDAR CHEESE AGING

ABSTRACT

Cooling of freshly formed Cheddar cheese is believed to be one of the processing steps requiring closer control to achieve more uniform and consistent flavor quality. Cheese samples obtained after pressing from a major local dairy processor were rapidly cooled to 12, 15, 20, and 25°C. After various days of aging, samples were evaluated for proteolysis, total acidity, pH, sugar and organic acid profile. Proteolysis and concentration of most organic acids increased as a function of time and temperature. Lactose disappearance and lactic acid production showed a non-significant temperature dependency. A kinetic analysis of organic acids production, changes in total acidity, lactose disappearance, and proteolysis extent generated parameters for theoretical and empirical equations for the effect of time and temperature on these chemical indexes. The application of these kinetic expressions to modelling of temperature control is also discussed.

INTRODUCTION

Cheese manufacture is essentially a dehydration process in which milk fat and casein are concentrated 6- to 10-fold depending on cheese variety (Fox, 1989). Cheese ripening is often a long, complicated and costly process. Its purpose is to convert the rubbery, relatively flavorless matrix of fresh pressed curd into a homogeneous mass recognizable as cheese with distinct aroma, taste, body, and texture. For Cheddar cheese, a period of at least 5 to 10 months is required, during which operating costs and interests on capital involved in cheese aging significantly add to the cost of production. The flavor of Cheddar cheese is ascribed to a complex mixture of chemical compounds produced by bacterial, chemical and enzymatic action during aging. Cheddar flavor originates from protein breakdown to simpler and more volatile products, lactic acid developed in the curd, milkfat, and the modest amount of salt added before the curd is pressed. In addition to metabolites produced from lactose fermentation, proteolysis and lipolysis are of significance in cheese flavor development. Cheese flavor intensity relates directly to the concentration of flavor components formed within the initial fermentation steps and the course of cheese aging (Bodyfelt et al., 1988). Several enzymes derived from the lactic starter culture and from the secondary microflora introduced during cheesemaking are responsible for these chemical changes (Bhowmik and Marth, 1990).

An important factor in producing cheese of uniform flavor quality is the extent of lactose utilization for lactic acid production in the vat stage, and also the subsequent microbial activity of residual starter culture and non-starter bacteria during cheese aging

(Gilles and Lawrence, 1973; Lawrence et al., 1983; Lawrence et al., 1984; Grazier et al., 1991b). Rapid cooling of cheese blocks to aging temperature is the primary means of control of this microflora activity and promotes homofermentative metabolism (Fryer, 1982). Quantitative comparisons of experimental samples with commercial samples from the same Cheddar cheese manufacturing plant showed as early as 7 and 30 days greater intensity of several characteristics in experimental cheese aged at higher temperatures as compared to commercial samples (Grazier et al., 1991a). Many of these sensory characteristics could be considered flavor defects because of their perceived intensity.

An early survey of Cheddar cheese manufacturers showed that a lack of control in the cooling step from pressing to curing was responsible for considerable variation in sensory characteristics within lots (Vedamuthu et al., 1969) and apparently this continue to be the case. Law et al. (1979) showed that the aging temperature was the most important factor in determining Cheddar cheese flavor intensity. The rate of cooling of cheese immediately after manufacture has been shown to be a factor affecting off-flavor development during aging (Fryer, 1982). Conochie and Sutherland (1965) found a correlation between occurrence of cheese flavor defects and uneven cooling of blocks of Cheddar closely stacked on pallets. Miah et al. (1974) studied the effects of four pressing/cooling treatments on flavor defects. They found a higher incidence of off-flavors associated with slower cooling rates. Aston et al. (1985) studied the effect of aging temperature (6, 13°C) on flavor preference. They concluded that higher aging temperature led to the production of strong off-flavors and lower sensory preference scores.

Production of glycolytic, proteolytic, and lipolytic metabolites increase with temperature, which may explain why high aging temperature often leads to flavor imbalances (Marsili, 1985). Elevated temperature conditions favor the earlier die-off of starter bacteria prior to complete lactose utilization and open the way for formation of off-flavors such as excessive sourness. The heterofermentative metabolism of lactose by non-starter bacteria produce by-products such as formic acid, ethanol and acetic acid (Law, 1984). Excessive levels of these compounds can impair the flavor balance of Cheddar cheese (Moskowitz, 1980). On the other hand, propionic and acetic acid concentrations increased significantly with aging temperature and were found to be extremely good predictors of Cheddar cheese age (Marsili, 1985). Short chain free fatty acids (C_4 to C_{10}) have been associated with lipolytic activity of adventitious bacteria although the role of these acids in Cheddar cheese flavor is still not well understood (Aston and Dulley, 1982). Proteolysis has been generally recognized as an acceptable indicator of cheese aging with the concentration of free amino acids and amines correlating significantly with flavor development (Puchades et al., 1989). Incomplete proteolysis could lead to less acceptable cheese due to the development of bitter flavor caused by peptides.

The purpose of the present study was to define and quantify the effect of time and temperature, varied along the general range of processing conditions, on selected chemical indexes. Analysis included sugar and organic acid profiles, the extent of proteolysis, pH, total acidity, and initial composition. A kinetic evaluation for the indicator variables during the aging period was also performed. In parallel studies, the same samples were evaluated for their sensory and microbial characteristics (Grazier et al., 1991a,b).

MATERIALS AND METHODS

Reagents

Sugars (glucose, galactose, and lactose), organic acids (citric, orotic, pyruvic, lactic, uric, formic, acetic, propionic, isovaleric, hippuric, and butyric), glycine, sodium tetraborate, and trinitrobenzenesulfonic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Standard sodium chloride solution was purchased from Orion Research Incorporated (Boston, MA). All solutions were prepared with distilled water purified by a Milli-Q system (Millipore, Bedford, MA).

Sample collection and storage

Samples of Cheddar cheese were obtained from the production of a commercial cheese manufacturer (Tillamook County Creamery Assn., Tillamook, OR) directly after the pressing operation. Cheddar cheese produced at this plant is made from flash-heated milk (65°C, 35s). Eighteen kg (40 lb) blocks from the same vat lot were cut into 2.5cm x 5cm x 5cm pieces. Each piece was vacuum shrink-wrapped in commercial O_2-barrier cheese film. Samples were placed in incubators at 12, 15, 20, and 25°C. The total time elapsed from cutting to equilibration at incubator temperature was less than 5 hours. Samples were randomly assigned to the four storage temperatures and tested at different stages during a three month period. The four batches used for testing were collected one week apart during June 1989.

HPLC analysis

Concentration of sugars and organic acids was evaluated following a modification of the procedure described by Bouzas et al. (1991a). The Shimadzu liquid chromatography system used (Shimadzu Scientific Instruments, Columbia, MD) consisted of a LC-6A solvent delivery unit, a RID-6A refractive index (RI) detector, a SPD-6AV variable wavelength UV/Visible detector, a CTO-6A column oven and a CR501 Chromatopac data processor. Under the reported chromatographic conditions, lactose and citric acid coeluted in a non-additive manner when peak heights were used. Peak areas were found to be additive and were therefore preferred to peak heights in this study. Citric acid RI peak area was estimated using UV data and citric acid calibration response data for the RI detector. The lactose signal was then determined by subtracting calculated citric acid area from total peak area.

Sample preparation. Twenty five mL of 0.009N H_2SO_4 (mobile phase) was added to 5 g of ground Cheddar cheese and extracted for 1 h while mixing with a magnetic stirrer. The extract was centrifuged at 5,000xg for 10 min. The supernatant was initially filtered through Whatman #1 filter paper and then through a 0.20 μm membrane filter (Bio-Rad Laboratories, Richmond, CA). Duplicate analyses were performed for all samples.

Proteolysis

The extent of proteolysis was evaluated by measuring free amino groups using trinitrobenzenesulphonic acid (TNBS) (Kuchroo et al., 1983; Polychroniadou, 1988.

Duplicate 1 g cheese samples were dispersed in 20 mL borate buffer (0.1M $Na_2B_4O_7$, pH 9.5), warmed at 45°C for 15 min with stirring, and then centrifuged at 3,000xg for 20 min. A 3 mL aliquot of the supernatant was diluted to 100 mL. A 0.5 mL portion of this extract was added to 0.5 mL borate buffer. Next, one mL TNBS reagent (1 mg/mL) was added. After thorough mixing, the solution was incubated at 37°C for 60 min. Blanks were prepared with 0.5 mL of H_2O instead of cheese extract. The reaction was stopped by adding 2 mL of 0.1M NaH_2PO_4 containing 1.5 mM Na_2SO_3 and the absorbance was measured at 420 nm. Glycine solutions were used as standards. A linear relationship was obtained between glycine concentration and color yield (r^2 = 0.9999).

pH and total acidity

A microprocessor pH/millivolt meter (Model 811; Orion Research Inc., Cambridge MA) with a combination spear-tip electrode (Model 91-63; Orion Research Inc.) was used for pH measurements. An X-shaped hole was cut into cheese samples and the electrode was inserted always to a depth of 18 mm. Measurements were conducted in triplicate.

Total acidity was determined using the AOAC official method (AOAC, Official Method 16.276, 1984) and the results were expressed as g lactic acid/100 g cheese.

Sodium chloride

Approximately 1 g of grated cheese was placed in a 250 ml beaker and 100 ml of 0.07 M nitric acid added. The beaker was covered with a watch glass and placed on a

stirrer/hot plate, heated until the boiling point was reached and gently boiled for 20 minutes. The beaker was then removed from the hot plate and cooled to room temperature. The contents were brought to 100 mL total volume in a volumetric flask. The sodium chloride content was determined using a chloride electrode (Model 94-17, Orion Research Inc.) with a double junction reference electrode (Model 90-02, Orion Research Inc.) connected to a microprocessor pH/millivolt meter (Model 811; Orion Research Inc.). Standard solutions (50, 100, 150, 200 ppm) were prepared by diluting a 1,000 ppm standard sodium chloride solution (Orion Research Inc.). Before sample evaluation, a calibration curve, millivolt reading vs. sodium chloride concentration (ppm), was prepared.

Proximate analysis

Duplicate moisture determinations were made on 2 to 3 g ground cheese samples and calculated as percent weight loss following drying for 4 h at 100±2°C in a vacuum oven (AOAC, Official Method 16.259, 1984). Duplicate fat determinations were conducted on 10 g ground cheese samples using the Roese-Gottlieb test (AOAC, Official Method 16.284, 1984). Duplicate protein determinations were made on 0.5 g ground cheese samples using the macro-Kjeldahl method (AOAC, Official Method 16.274, 1984).

Statistical analysis

An analysis of variance was performed on the data with temperature, time and batch as main effects with temperature and time treated as fixed effects. Batch, viewed

as a representative sampling from a population, was treated as a random effect (Grazier et al., 1991a). Estimates of mean square values were generated using the General Linear Model procedure for unbalanced data on the SAS statistical software package (Version 6.02, SAS Inc., Cary, NC). The objective was to study the effects of temperature and time concurrently. For that reason, all indicator variables were tested for batch, time, temperature effects and the interaction of time and temperature using the GLM procedure for unbalanced data on the entire data set. When time by temperature interaction was significant ($P < 0.05$) the results were summarized in a two way table of means (Petersen, 1985). LSD values were used to determine differences between the means. If interaction was not significant, all information was contained in the main effects, time and temperature. In this case, the results were summarized in a table of means for time and temperature (Petersen, 1985).

Nonlinear regression analysis, using the SAS software package, was conducted on the entire data set to obtain the coefficients for the different terms of the kinetic equations for the indicator variables. Residual analysis was used to determine the degree of fitness of the equations obtained (Neter et al., 1989; Thomas, 1990).

RESULTS AND DISCUSSION

Proximate analysis

It has been suggested that to produce Cheddar cheese of the most acceptable flavor quality, initial composition should fall within a certain range (Turner and Thomas, 1980; Lawrence and Gilles, 1980; Lawrence et al., 1984). It is important to recognize,

however, that the absolute values for fat, moisture and salt are not in themselves of key importance. The more relevant parameters are fat-in-dry-matter (FDM), moisture in the non-fatty substance (MNFS) and salt-in-moisture (S/M) (Lawrence and Gilles, 1980). The composition ranges for the Cheddar cheese examined in our study along with the optimal range for premium cheese as suggested by Lawrence et al. (1984) are presented in Table V.1. Values were within the premium cheese range except for FDM which is slightly above the recommended level.

Time and temperature effects

Statistical significance of the effects of time, temperature, and time by temperature on the indicator variables chosen for this study are presented in Table V.2. Time by temperature interaction was significant ($P < 0.05$) for all characteristics except lactose, lactic acid and total acidity (Table V.2). Time and temperature main effects were significant for total acidity but the interaction was not significant. Lactose and lactic acid showed only a statistically significant time effect.

Sugars and lactic acid. The proportion of residual (unmetabolized) lactose in Cheddar curd at the beginning of the cooling process was about 2 mmoles/100 g cheese. Starter bacteria are not totally inhibited by the salting process and this microflora could continue to metabolize lactose (Lawrence et al., 1984) In addition, non-starter bacteria such as lactobacilli and pediococci affect the rate of lactose metabolism. The homolactic fermentation of residual lactose by starter and non-starter cheese microflora has been

directly associated with Cheddar cheese quality (Fryer, 1982). It leads to the production of lactic acid by the hexose diphosphate pathway. Residual lactose fermentation and lactic acid production at 12 and 25°C for the four batches under study are shown in Figures V.1 and V.2. Similar results were observed for the samples stored at 15 and 20°C. Negligible amounts of lactose were detected after 10 days for the four batches and four aging temperatures. This is in agreement with many other researchers which have reported that lactose is utilized rather early in the aging of Cheddar cheese, generally within the first two weeks of curing (Miah et al., 1974). In most samples free galactose was detectable at very low levels (<0.05 mmoles/100 g) while no free glucose was found. This finding served to confirm, as generally acknowledged, that glucose is the preferred microbial substrate of the two lactose hydrolysis end products.

No temperature effect was observed for lactose consumption and lactic acid production. The F-value for the effect of temperature on lactose utilization and lactic acid production showed no significance ($P = 0.22$ and 0.16, respectively). Consistent results were observed for the four batches used in this study. On the other hand, a statistically significant effect of time was found ($P < 0.05$). This is consistent with the parallel study by Grazier et al. (1991a) who found sourness increasing most readily during the early stages of aging.

It should be noted that Turner and Thomas (1980) reported an effect of temperature on lactose fermentation. A difference in experimental procedures and a probable difference in cheese microflora could be responsible for the discrepancy in results. Jensen et al. (1975) reported significant differences in lactic acid production for

Cheddar cheese made with different strains of lactococci. Also, Grazier et al. (1991b) showed in a parallel study that both growth of non-starter lactic acid bacteria and die-off of starter culture were accelerated by temperature. Thus, the observed lack of temperature effect on lactose fermentation and lactic acid production could reflect the consequence of the opposite effect of temperature on non-starter lactic acid bacteria and starter culture.

Grazier et al. (1991b) suggested that microbial activity during Cheddar cheese aging may be controlled by diffusion of nutrients (e.g. lactose) to the cell or metabolites (e.g. lactic acid) away from the cell. A lower cheese temperature would extend the time of starter culture viability by allowing time for diffusion of slowly produced lactic acid away from the starter bacteria which would inhibit the growth of non-starter lactic acid bacteria. A higher cheese temperature would lead to rapid lactic acid production by the starter bacteria which would inactivate them before consumption of all available lactose. The remaining lactose would be consumed by non-starter lactic acid bacteria which would produce other metabolites in addition to lactic acid.

Citric, acetic, and propionic acids. The interaction of time and temperature for citric acid was statistically significant ($P < 0.05$). The citric acid disappearance rate was higher with successively higher temperature (Table V.3). For the four temperatures there were no statistically significant differences up to day four. Starting with day eight we found significant differences, with 12 and 25°C samples being different from the other two. Elevated storage temperature is associated with atypical growth patterns of non-

starter lactic acid bacteria. Deviations by homofermentative lactic acid bacteria from homolactic fermentation have been extensively described (Fryer, 1982; Fordyce et al., 1984; Tellow and Hoover, 1988; Laleye et al., 1990).

Heterofermentative lactobacilli metabolize citrate, or have induced NADH oxidase activity, and produce acetic acid, ethanol and various carbonyl compounds (Aston and Dulley, 1982; Laleye et al., 1990). Strains of *Lactobacillus casei*, *L. plantarum* and *L. brevis* are capable of using citrate for energy in the absence of carbohydrate (Fryer, 1970; Peterson and Marshall, 1990). Harvey and Collins (1963) showed that *Lactococcus lactis* subsp. *diacetylactis* was unable to use citrate as a source of energy for growth, but can metabolize it showing an increase of 35% in specific growth rate. These authors postulated that citrate allowed the synthesis of a cell component that was produced only very slowly in its absence. Citrate was converted to pyruvate which was then used to generate the cell component with excess pyruvate used to produce acetoin.

Production of acetic and propionic acids in addition to lactic acid contributed to the overall sourness of the Cheddar cheese samples under study as reported by Grazier et al. (1991a). The interaction of temperature and time on acetic and propionic acid production was significant ($P < 0.05$). Propionic and acetic acid production rate increased with temperature confirming previous results reported by Marsili (1985). No significant differences among temperatures were observed up to days four and eight for acetic and propionic acid, respectively (Tables V.4 and V.5). Samples aged at 25°C became different from those stored at the other three temperatures by day eight for acetic acid and day 16 for propionic acid. All four temperatures were significantly different by

day 48. Acetic acid, which can be produced from citrate, lactose and amino acids (Aston and Dulley, 1982), provides an indication of the degree of heterofermentative metabolism that may have taken place (Lawrence and Gilles, 1987). More rapid growth of non-starter bacteria encourages heterolactic fermentation of lactose.

Propionate is a major end product of fermentations carried out by a variety of anaerobic bacteria. Propionibacteria and anaerobic sporeformers (clostridia strains) can survive heat treatment or be present in the dairy plant environment and contaminate the curd. Propionate production has been attributed to this non-starter contamination (Sandine, 1991). Another possibility could be propionate production from casein amino acids which have been reported to be a significant source of volatile fatty acids (acetic to caproic) (Nakae and Elliot, 1965; Dulley and Grieve, 1974).

Butyric and isovaleric acids. It is generally thought that lipases from milk, starter and non-starter bacteria are important during cheese aging (Bhowmik and Marth, 1990). Lipases hydrolyse low molecular weight fatty acids from milkfat in a non selective manner (Lamparsky and Klimes, 1981). The most important low molecular weight fatty acid formed by this mechanism is butyric acid which contributes to the aroma and flavor of Cheddar cheese. In a parallel study, butyric acid was selected by the trained panel as a standard for the goaty-like aroma and flavor observed (Grazier et al., 1991a).

Table V.6 shows the concentration changes of butyric acid with time and temperature. We found no obvious trend in butyric acid production up to day 32. From day 48 on, samples stored at all temperatures were significantly different with an increase

in production rate with higher temperature. Intracellular lipases liberated during the die-off of starter bacteria could be responsible for this behavior. Accelerated die-off of starter bacteria with increasing aging temperature can be expected to contribute to the development of butyric acid concentration. On the other hand, butyrate production could be accounted for by other mechanisms. Aston and Dulley (1982) reported that free fatty acids from butyric to caproic were produced in Cheddar cheese slurries manufactured from milk whose fat had been replaced by vegetable lipids. These lipids contained no fatty acids of chain length less than C_{12} thus eliminating lipolysis as a direct source. Also, Nakae and Elliot (1965) demonstrated that free fatty acids from acetic to caproic were produced from casein hydrolysates by lactic acid bacteria.

Isovaleric acid production with time and temperature is shown in Table V.7. The rate of production increased dramatically with temperature, leading to significant differences at day 2, 4, 20, and 48 for the samples stored at 25, 20, 15, and 12°C, respectively. In a concurrent study, Grazier et al. (1991a) used isovaleric acid as a trained panel standard for dirty-like aroma and aftertaste in Cheddar cheese.

Stadhouders and Veringa (1973) suggested that longer chain fatty acids ($> C_4$) were produced through deamination of amino acids. Lamparsky and Klimes (1981) assumed that isovaleric acid originated from the corresponding amino acid (valine) via deamination since milkfat does not contain glycerides with the isovaleric acid moiety. Puchades et al. (1989) showed that valine was one of the principal amino acids liberated through proteolytic activity of starter and non-starter bacteria and that an increase in temperature from 6 to 15°C intensified proteolysis. Some strains of lactobacilli have been

associated with a reduction of valine levels during later stages of aging. Puchades et al. (1989) suggested that this microbial activity is associated with the formation of deamination products (i.e., isovaleric acid).

Pyruvic, orotic and hippuric acids. Pyruvic acid, whose production did not follow any particular trend, is a key component for the production of further flavoring materials. This behavior could be attributed to its characteristic as an intermediate in several metabolic pathways. Orotic acid is commonly present in ruminants milk and is part of the soluble whey constituents. A decrease in orotic acid amounts was observed but with no specific trend (data not shown). Orotic acid has been reported to be utilized by various bacteria during fermentation of dairy products (Chen and Larson, 1971; Empie and Melachouris, 1977; Larson and Hegarty, 1979). Consistent with the results by Marsili et al. (1985), negligible amounts of hippuric acids were detected.

Proteolysis. Rate of proteolysis increased with an increase in temperature leading to the samples stored at 25°C being significantly different from the other three by day 16 and all four temperature treatments being significantly different by day 32 (Table V.8). This is in agreement with the results by Grazier et al. (1991a), who reported significant differences in bitter taste for all temperatures starting at 30 days.

Rennet is largely responsible for the hydrolysis of casein to non-bitter, high molecular weight peptides. Intracellular peptidases released during the die-off of starter bacteria reduce these products to bitter, low molecular weight peptides (O'Keefe et al.,

1976; Lawrence et al., 1983). Accelerated die-off of starter bacteria with increasing aging temperature (Grazier et al., 1991b) can be expected to contribute to an increase in proteolytic activity, i.e. development of more bitter taste. In addition, non-starter bacteria, particularly the lactobacilli, contribute to proteolysis by conversion of proteins into small peptides (Reiter et al., 1969; O'Keefe et al., 1976; Law and Sharpe, 1977; Desmazeaud and Gripon, 1977; Lee et al., 1990a). Lee et al. (1990b) found that several lactobacilli added to starter culture resulted in more bitterness in cheese as compared to cheese made only with *Lactococcus spp.* starter culture.

Total acidity and pH. Total acidity exhibited two main effects, time and temperature but the interaction of time and temperature was not significant. This indicates there is no difference in the rate of acidity development among temperature treatments over the total aging period. Total acidity values are reported in Table V.9 as mean values to observe the general evolution trend of acid production. Significant differences were found for all treatments with total acidity increasing with increasing temperature and time (Table V.9) reflecting the production of organic acids previously described.

The pH variation with time and temperature shows a marked pattern of decrease and later increase in pH for all storage temperatures except for samples aged at 12°C which showed a negligible pH change (Figure V.3). The initial decrease in pH can be attributed to organic acid production and the subsequent increase to the formation of basic compounds, i.e., amino acids and amines. The extent of decrease and increase in pH was markedly affected by temperature.

Commercial cheese analysis

Commercial mild Cheddar cheese samples supplied by the manufacturer of the experimental cheese and aged for 60 days at 7°C were also analyzed. A formal study of the characteristics of commercial Cheddar was not consistent with the objective of this research, however, the values shown in Table V.10 for commercially acceptable cheese were helpful in the relative evaluation of the experimental samples. Our analysis indicated that cheeses stored at higher temperatures quickly reach concentration levels in excess of the ones found in the commercial samples for most of the parameters of interest. This finding reinforces previous reports (e.g. Lawrence and Gilles, 1980; Lawrence et al., 1984) on the importance of cooling rate and aging temperature on the final flavor quality of Cheddar cheese.

Kinetic studies

A kinetic analysis of the chemical indexes found to change significantly with time and temperature was conducted to develop kinetic equations based on experimental data statistically fitted to empirical, or whenever possible, to theoretical expressions. The temperature effect was quantified using the Arrhenius model except for lactose and lactic acid where temperature dependency was not observed. Final expressions are summarized in Table V.11.

Citric acid and lactose. Disappearance of these two components was found to follow an unimolecular and irreversible first order reaction kinetic model, A $\rightarrow$ Products.

Lactic acid production and total acidity. These two indexes were also found to follow unimolecular and irreversible first order reactions.

Proteolysis. Protein breakdown, measured as concentration of α- and ε-amino groups, was considered to be pseudo zero order. The reasonably high amount of protein in cheese as a substrate for proteolysis justifies the modelling of this reaction as pseudo zero order kinetics (Levenspiel, 1972).

Acetic, propionic, butyric and isovaleric acids. These acids can be produced through a number of parallel and series reactions. Theoretical expressions for various assumed models based on two irreversible reactions, in parallel and in series, and of zero and first order kinetics, failed to fit the experimental data. Expressions developed by fitting experimental data to an exponential function, dependent on temperature and time, provided a reasonable fit to the experimental data.

A residual analysis was performed to examine the degree of fit of the models to the experimental data (Neter et al., 1989; Thomas, 1990). In the case of theoretically derived expressions (lactose, proteolysis, total acidity, and citric and lactic acids), the error terms were evenly distributed with no specific trend observed. The residual analysis for the empirical expressions showed error terms evenly distributed up to day 32 and thereafter the error terms increased with storage time.

The deviation of the experimental data from the predicted values, for the different chemical indexes, was estimated by calculating a relative error for each temperature. This

error was determined as the average, over the three months storage period, of the differences between predicted and experimental values divided by the average experimental value over the same period (Table V.12). The errors for total acidity, lactic and citric acid, are small considering that the samples were obtained from a commercial processor. As expected, the error terms for acetic, propionic, butyric and isovaleric acids are somewhat larger than the ones calculated for the other indexes due to the lack of fit for aging times larger than 32 days. In the case of lactose, the error is due to the difficult quantification of very small amounts of lactose by HPLC which for all practical purposes can be considered zero (Figure V.1).

CONCLUSIONS

This study reinforces previous reports on the importance of cooling rate and aging temperature on the final quality of Cheddar cheese. In general, the equations developed for all the chemical indexes correctly predict the effect of time and temperature and thus could be combined with heat transfer models to predict the effect of block size, and cooling and aging temperature modifications, on Cheddar cheese composition. Cheese composition could be related to sensory quality on the basis of reported flavor changes (e.g. Grazier et al., 1991a).

Table V. 1 Initial composition of the Cheddar cheese samples under study

Index [1]	Range [2]	Batch # 1	2	3	4
S/M [3]	4.0 - 6.0	5.6	6.0	5.3	5.8
MNFS [3]	52 - 56	55	56	55	56
FDM [3]	52 - 55	57	56	58	57
pH [4]	4.9 - 5.1	4.9	5.1	5.1	5.1
Protein % [3] (dry basis)	--------	40	40	39	40

(1) S/M, salt-in-moisture; MNFS, moisture-in-non-fat-solids; FDM, fat-in-dry matter
(2) Recommended values by Lawrence and Gilles (1987)
(3) Mean of duplicate determinations
(4) Mean of triplicate determinations

Table V. 2 Significance levels for Cheddar cheese descriptors [1]

Acids	A	T	A x T
Citric	***	***	***
Lactic	***	NS	NS
Acetic	***	***	***
Propionic	***	***	***
Butyric	***	***	***
Isovaleric	***	***	***
Sugars			
Lactose	***	NS	NS
Proteolysis	***	***	***
Total acidity	***	***	NS

(1) A = age; T = temperature; *** = $P < 0.05$; NS = non-significant

Table V. 3 Means for the interaction of temperature and time (age) on citric acid concentration (mmoles/100 g cheese, dry basis) [1, 2]

	Temperature (°C)				
Time days	12	15	20	25	LSD
0	1.15^{cd1}	1.15^{de1}	1.15^{de1}	1.15^{ef1}	0.07
2	1.16^{cd2}	1.11^{d12}	1.08^{d1}	1.08^{e1}	
4	1.20^{d1}	1.19^{ef1}	1.22^{e1}	1.17^{f1}	
8	1.22^{d23}	1.26^{f3}	1.18^{e2}	1.00^{d1}	
16	1.22^{d3}	0.99^{c2}	0.92^{c2}	0.67^{c1}	
20	1.10^{bc4}	1.00^{c3}	0.90^{c2}	0.64^{c1}	
32	1.04^{b3}	0.86^{b2}	0.83^{b2}	0.42^{b1}	
48	0.90^{c3}	0.85^{b23}	0.81^{b2}	0.36^{b1}	
99	0.79^{a4}	0.72^{a3}	0.55^{a2}	0.13^{a1}	

(1) a → f: means with the same letter are not significantly different within temperatures
(2) 1 → 4: means with the same number are not significantly different within days

Table V. 4 Means for the interaction of temperature and time (age) on acetic acid concentration (mmoles/100 g cheese, dry basis) [1, 2]

Time days	Temperature (°C) 12	15	20	25	LSD
0	0.30^{a1}	0.30^{a1}	0.30^{a1}	0.30^{a1}	0.30
2	0.28^{a1}	0.26^{a1}	0.27^{a1}	0.30^{a1}	
4	0.37^{a1}	0.25^{a1}	0.42^{a1}	0.36^{ab1}	
8	0.34^{a12}	0.36^{a12}	0.29^{a1}	0.62^{b2}	
16	0.30^{a1}	0.30^{a1}	0.36^{a1}	1.20^{c2}	
20	0.42^{a1}	0.63^{b1}	0.77^{b1}	1.80^{d2}	
32	0.47^{a1}	1.03^{c2}	1.11^{c2}	2.77^{e3}	
48	0.48^{a1}	0.97^{c2}	1.63^{d3}	4.44^{f4}	
99	1.12^{b1}	2.64^{d2}	4.40^{e3}	7.07^{g4}	

(1) a → g: means with the same letter are not significantly different within temperatures
(2) 1 → 4: means with the same number are not significantly different within days

Table V. 5 Means for the interaction of temperature and time (age) on propionic acid concentration (mmoles/100 g cheese, dry basis) [1, 2]

	Temperature (°C)				
Time days	12	15	20	25	LSD
0	0.21^{a1}	0.21^{a1}	0.21^{a1}	0.21^{a1}	0.55
2	0.21^{a1}	0.24^{a1}	0.24^{a1}	0.26^{a1}	
4	0.24^{a1}	0.23^{a1}	0.35^{a1}	0.24^{a1}	
8	0.26^{a1}	0.32^{a1}	0.30^{a1}	0.43^{a1}	
16	0.26^{a1}	0.42^{a1}	0.55^{a1}	1.23^{b2}	
20	0.38^{a1}	0.38^{a1}	1.55^{b2}	2.60^{c3}	
32	0.30^{a1}	1.29^{b2}	1.47^{b2}	3.75^{d3}	
48	0.60^{a1}	1.53^{b2}	2.80^{c3}	5.60^{e4}	
99	$2,35^{b1}$	4.46^{c2}	8.84^{d3}	19.90^{f4}	

(1) a → f: means with the same letter are not significantly different within temperatures
(2) 1 → 4: means with the same number are not significantly different within days

Table V. 6 Means for the interaction of temperature and time (age) on butyric acid concentration (mmoles/100 g cheese, dry basis) [1, 2]

	Temperature (°C)				
Time days	12	15	20	25	LSD
0	0.16^{a1}	0.16^{a1}	0.16^{a1}	0.16^{a1}	0.13
2	0.16^{a1}	0.16^{a1}	0.47^{bc2}	0.21^{bc1}	
4	0.27^{ab1}	0.38^{b12}	0.53^{c3}	0.42^{d23}	
8	0.29^{bc1}	0.36^{b1}	----	0.36^{d1}	
16	0.24^{ab1}	----	0.23^{a1}	0.33^{cd1}	
20	0.29^{bc1}	0.44^{bc2}	0.40^{b12}	0.69^{c3}	
32	0.34^{bc1}	0.36^{b1}	0.36^{b1}	----	
48	0.28^{bc1}	0.42^{bc2}	0.55^{c3}	1.20^{f4}	
99	0.40^{c1}	0.54^{c2}	0.80^{d3}	1.74^{g4}	

(1) a → g: means with the same letter are not significantly different within temperatures
(2) 1 → 4: means with the same number are not significantly different within days

Table V. 7 Means for the interaction of temperature and time (age) on isovaleric acid concentration (mmoles/100 g cheese, dry basis) [1, 2]

Time days	Temperature (°C) 12	15	20	25	LSD
0	0.34^{a1}	0.34^{a1}	0.34^{a1}	0.34^{a1}	0.24
2	0.39^{ab1}	0.44^{ab1}	0.42^{ab1}	0.71^{b2}	
4	0.43^{ab1}	0.43^{ab1}	0.74^{c2}	0.70^{c2}	
8	0.35^{a1}	0.64^{bc2}	0.60^{c2}	0.73^{bc2}	
16	0.62^{b1}	0.55^{ab1}	0.61^{bc1}	0.96^{cd2}	
20	0.47^{ab1}	0.84^{cd2}	1.02^{d2}	1.29^{d3}	
32	0.56^{ab1}	1.07^{d2}	1.07^{d2}	2.03^{e3}	
48	0.60^{b1}	0.97^{d2}	1.70^{e3}	2.89^{f4}	
99	1.44^{c1}	2.83^{e2}	3.74^{f3}	4.77^{g4}	

(1) a → g: means with the same letter are not significantly different within temperatures
(2) 1 → 4: means with the same numbers are not significantly different within days

Table V. 8 Means for the interaction of temperature and time (age) on proteolysis extent (mmoles glycine/100 g cheese, dry basis) [1, 2]

	Temperature (°C)				
Time days	12	15	20	25	LSD
0	19.0^{a1}	19.0^{a1}	19.0^{a1}	19.0^{a1}	13
2	24.3^{ab1}	26.0^{a1}	27.7^{ab1}	30.0^{a1}	
4	25.0^{ab1}	28.0^{a1}	35.0^{bc1}	32.0^{b1}	
8	35.5^{bc1}	42.2^{b1}	42.7^{c1}	48.2^{c1}	
16	47.7^{cd1}	51.7^{b1}	59.7^{d1}	77.7^{d2}	
20	55.0^{d1}	55.3^{c1}	66.3^{d1}	81.7^{d2}	
32	55.3^{d1}	81.7^{d2}	95.0^{e3}	125.7^{e4}	
48	70.0^{e1}	101.0^{e2}	122.0^{f3}	173.4^{f4}	
99	191.0^{f1}	225.3^{f2}	234.7^{g2}	301.3^{g3}	

(1) a → g: means with the same letter are not significantly different within temperatures
(2) 1 → 4: means with the same number are not significantly different within days

Table V. 9 Main effects of time and temperature on total acidity (g lactic acid/100 g cheese, dry basis)

Temperature (°C)	Total Acidity	LSD
12	1.34[a]	0.02
15	1.44[b]	
20	1.50[c]	
25	1.55[d]	

Time (days)	Total acidity	LSD
0	1.09[a]	0.03
2	1.23[b]	
4	1.36[c]	
8	1.40[d]	
16	1.45[e]	
20	1.51[f]	
32	1.66[g]	
48	1.75[h]	
99	1.95[i]	

(1) a → i: means with the same letter are not significantly different

Table V. 10 Composition of commercial cheese [1] aged for 60 days at 7°C

Acids, mmoles/100 g d.b. [2]	Composition
Citric	1.00 ± 0.04
Lactic	36.52 ± 2.32
Acetic	0.38 ± 0.05
Propionic	0.37 ± 0.08
Butyric	0.16 ± 0.03
Isovaleric	0.45 ± 0.06
Sugars, mmoles/100 g d.b.	
Lactose	0.085 ± 0.042
Glucose	ND [3]
Galactose	ND
Proteolysis, mmoles glycine/100 g d.b.	124.6 ± 6.1
Total Acidity, g lactic acid/100 g d.b.	1.48 ± 0.08

(1) Mean of 5 replicates
(2) d.b. = dry basis
(3) ND = non detectable

Table V. 11 Kinetic equations

Zero Order	Component	Coefficients
$Y = Y_o + k_o\, t \exp(b/T)$	Proteolysis	$k_o = 1.79\ 10^8$ $b = -5315$
First Order		
$Y = Y_o \exp(k\, t)$	Lactose	$k = -0.3194$
$Y = Y_o \exp(k_o\, t \exp(b/T))$	Citric acid	$k_o = -1.09\ 10^{16}$ $b = -12108$
$Y = Y_o + A\,(1 - \exp(k\, t))$	Lactic acid	$A = 2.87$ $k = -0.2848$
$Y = Y_o + A\,(1 - \exp k_o\, t\,(\exp(b/T))$	Total acidity	$A = 0.7651$ $k_o = -4.39\ 10^9$ $b = -7402$
Mathematical Fitting		
$Y = Y_o \exp(a\, t \exp(c/T))$	Acetic acid	$a = 5.21\ 10^{10}$ $c = -8222$
	Propionic acid	$a = 1.8\ 10^{11}$ $c = -8502$
	Butyric acid	$a = 1.64\ 10^{12}$ $c = -9407$
	Isovaleric acid	$a = 4.5\ 10^8$ $c = -6831$

Y_o = initial concentration, lactose & acids in mmoles/100 g, proteolysis in mmoles glycine/100 g, total acidity in g lactic acid/100 g
T = absolute temperature in K; t = time in days; k_o(0 order) = concentration/time
k_o(1st order) = 1/time; b in K; k = 1/time; A = concentration; c in K; and, a = 1/time.

Table V. 12 Relative errors for the prediction of the chemical indexes

	Relative error %[1]			
	Temperature (°C)			
Compound	12	15	20	25
Citric acid	8	12	11	12
Lactic acid	6	6	7	6
Acetic acid	31	39	35	27
Propionic acid	30	32	26	17
Butyric acid	45	42	44	43
Isovaleric acid	24	32	33	22
Lactose	25	21	24	31
Proteolysis	17	16	19	14
Total acidity	9	8	7	6

(1) Calculated as $1/n \, \Sigma_{time} \, | Y_{predicted} - Y_{experimental} | \, / \, 1/n \, \Sigma_{time} \, Y_{experimental}$

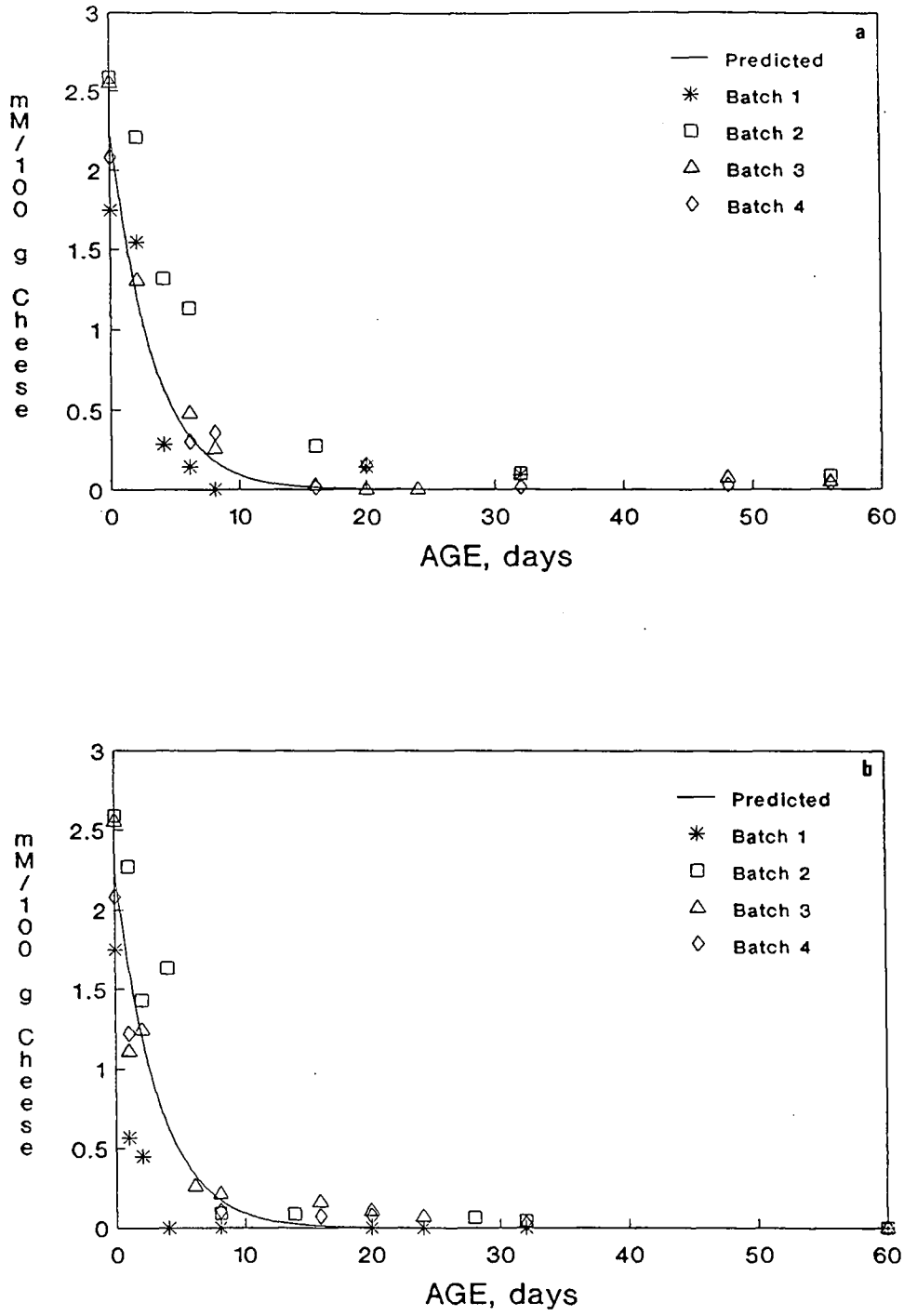

Figure V. 1 Changes in lactose concentration in Cheddar cheese samples during aging: (a) 12°C and (b) 25°C

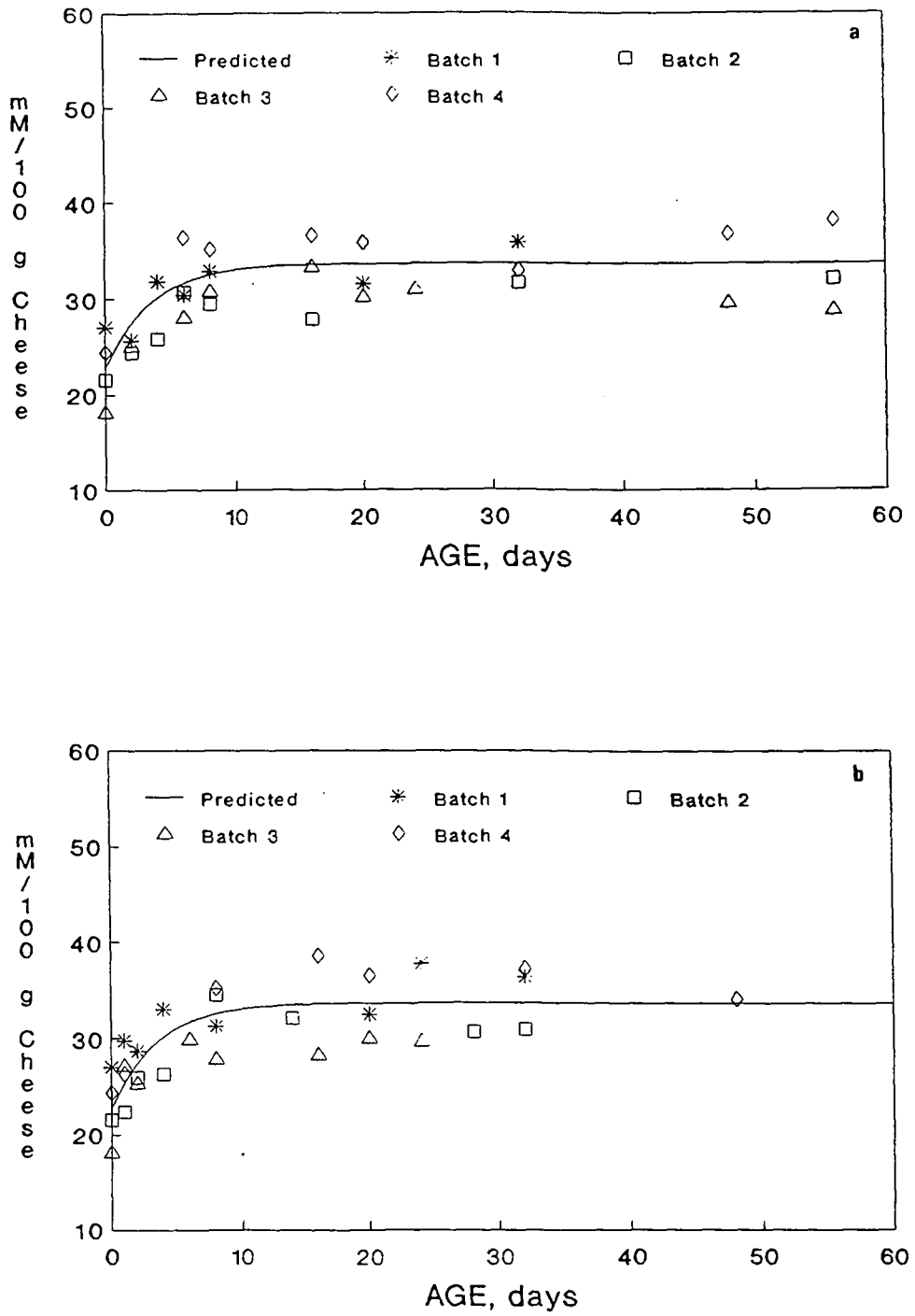

Figure V. 2 Changes in lactic acid concentration in Cheddar cheese samples during aging: (a) 12°C and (b) 25°C

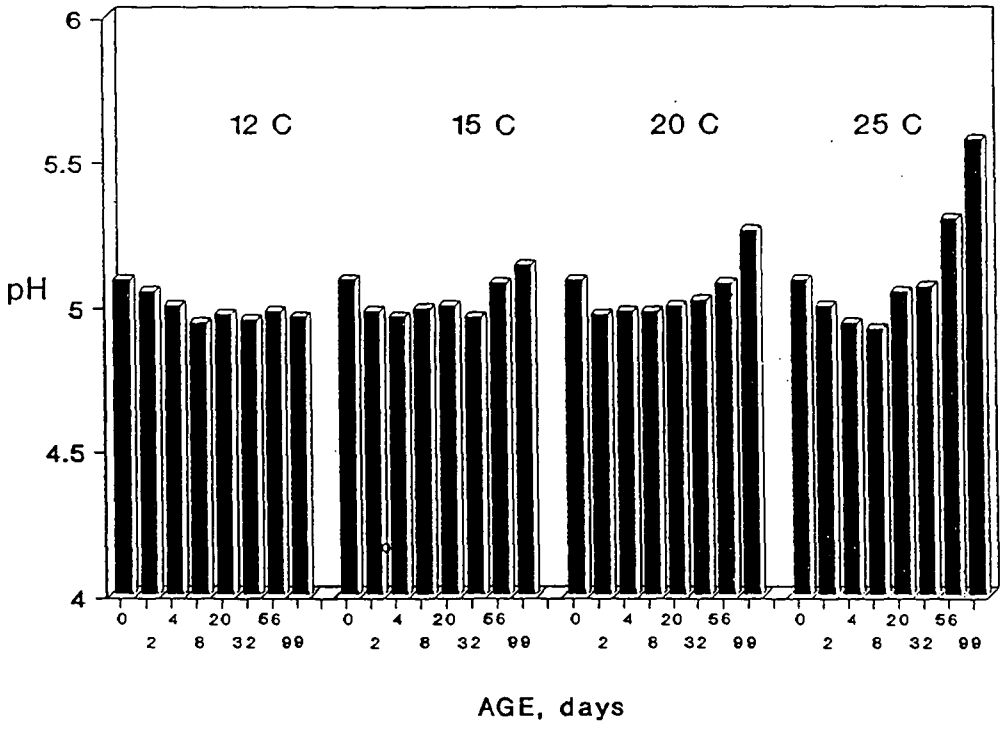

Figure V. 3 Cheddar cheese pH changes during aging at four different storage temperatures: 12, 15, 20, and 25°C

VI. APPLICATION STUDIES

The experimental results obtained in this study and the ones reported in parallel studies by Grazier et al. (1991a,b) provide information on Cheddar cheese aging at constant and homogeneous temperature conditions. This is not the case of a commercial Cheddar cheese block, particularly during the initial cooling period. Therefore, a heat transfer model was needed to predict temperature as a function of time and location within a cheese block. This information was combined with kinetic expressions for the effect of time and temperature on flavor quality. The method for heat transfer calculations has general validity. However the parameters in the equations used were quantified for the particular case of the dairy plant where the commercial samples were obtained (Tillamook County Creamery Association, Tillamook, OR) (Torres et al., 1991).

In addition to the expressions obtained in this study for chemical changes with time and temperature, kinetic equations were also derived for selected sensory indexes following the methodology described in this thesis (Torres et al., 1991). The mathematical model for heat transfer was based on a finite difference approximation of the differential equation for transient heat conduction in three dimensions. A numeric calculation procedure was selected for its flexibility when handling changing environmental and boundary conditions (Torres et al., 1991). In our case, it was necessary to estimate convective heat transfer coefficients (Kreith, 1976) associated with the air velocity and temperature which was different for the cooling chamber and the aging room. Convective heat transfer limitations were incorporated by introducing an

overall heat transfer coefficient defined as:

$$U = 1/[1/h + \Sigma(\delta_i / k_{P,i})]$$

where h = convective heat transfer coefficient, $k_{P,i}$ = thermal conductivity of the wrapping materials, and δ_i = thickness of the packaging materials (Zuritz and Sastry, 1985). The experimental determination of this overall heat transfer coefficient was done considering that the cheese blocks are packaged in corrugated cardboard boxes with a cardboard liner placed on the vertical sides of the box. Therefore, four U-values were needed as summarized in Table VI.1 (Torres et al., 1991).

Calculated temperature distributions in the cheese block while in the cooling chamber, and also in the aging room, were used to predict sensory and chemical characteristics at a large number (>1,200) of locations within the block. A computer optimization method (Banga and Casares Long, 1987; Casares and Rodriguez, 1989) was used in conjunction with the heat transfer and kinetic models to determine cooling and aging conditions that reduce sensory and chemical differences between the various locations within the block. Furthermore, the search for temperature conditions was limited to those yielding predicted chemical and sensory indexes close to those obtained for commercial samples of desirable quality (Bouzas et al., 1991b). Restrictions were placed on both the set point temperatures (maximum-minimum values) and the time length in both the cooling chamber and the aging room. Table VI.2 shows the prediction for selected chemical and sensory indexes for a possible process at the Tillamook County

Creamery Association plant. It can be seen that with these process conditions, two chemical indexes and one sensory value are out of the range desired by the processor. The final objective of this research project is to determine process conditions that produce cheese with the largest number of acceptable values falling within the specified range.

Table VI. 1 Overall heat transfer coefficients

Cooling Chamber	U (J/s m^2 K)
Vertical side	3.5
Horizontal side	6.0
Aging Room	
Vertical side	1.3
Horizontal side	1.0

Table VI. 2 Effect of operational parameters on flavor quality indexes

Cooling & aging conditions

Cooling temperature:	1.1°C
Cooling time:	18 h
Aging temperature:	7°C
Aging time:	60 days

Index	Objective range Min - Max	Predicted range Min - Max	
Chemical	ppm	ppm	
Acetic Acid	180-350	280-288	
Butyric Acid	110-190	172-175	
Citric Acid	1,800-2,100	1,925-1,962	
Isovaleric Acid	380-570	566-577	
Lactic Acid	29,000-35,000	30,500-32,100	
Propionic Acid	230-380	344-357	
Total Acidity	1.4-1.6	1.97-1.99	⇐
Proteolysis	110-135	97-99	⇐
Lactose	0-500	0-0	
Sensory	0-15 Scale	0-15 Scale	
Overall Aroma	6.4-7.4	7.2-7.3	
Overall Flavor	6.9-7.4	7.0-7.1	
Sourness	4.9-5.8	5.5-5.6	
Bitterness	4.3-4.9	2.6-2.7	⇐

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