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 in the United States, Cheddar cheese. Cooling of freshly formed cheese is believed to be a processing step requiring closer control to achieve uniform and consistent flavor characteristics.

The effect of time and temperature on the sensory and microbiological characteristics during aging were studied using cheese samples from a major local processor. Samples were obtained after pressing and were rapidly cooled to 5, 15, 25, and 35°C. Retail medium aged samples from the same processor and test cheeses at 7, 30, 60, 90, and 120 d of ripening were evaluated by a trained descriptive panel. Most sensory characteristics of experimental cheeses increased in intensity as a function of the interaction of time and temperature. The perception of sour and salty taste were affected by temperature, and at equal rates over time. Buttery aroma and flavor tended to decrease in intensity as a function of time and temperature.
During aging, starter and non-starter bacteria contributed extensively to the flavor of Cheddar cheese. Temperature effects on these bacteria were quantified using duplicate samples from the sensory trials. At day 1, starter counts were $8 \times 10^7$ CFU/g but as aging continued, starter counts decreased and non-starter bacteria became dominant. At $35^\circ C$, starter counts reached $3 \times 10^6$ CFU/g by day 3 and were below $10^6$ CFU/g by day 5. At $25^\circ$, $20^\circ$, $15^\circ$ and $12^\circ C$, starter bacteria were below $10^6$ CFU/g by day 10, 20, 24 and 40, respectively. Non-starter counts, initially at $10^4$ CFU/g and including lactobacilli and pediococcus, reached $=10^8$ CFU/g at increasingly shorter times with higher temperatures and remained at low levels in samples stored at $5^\circ C$. 
Time-Temperature Effects on Cheddar Cheese Ripening:

Sensory and Microbiological Changes

by

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Much appreciation goes to the Western Dairy Research Center and Tillamook County Creamery Association for supplying the funds, materials and facilities without which no research would have been possible.

A standing ovation goes out to the members of my sensory panel for donating many hours of their precious time to the pursuit of our goals. Their tolerance of the several potent and objectionable cheese samples is a tribute to their professionalism.

Thanks goes out to family and friends for their support and encouragement. I dedicate this effort to my dear husband, Bill, whose devotion to helping me achieve a very personal goal made it happen. I will always be indebted to him. God bless his generous soul.
# TABLE OF CONTENTS

## I. INTRODUCTION

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

## II. LITERATURE REVIEW

- Cheddar cheese technology .................................................. 7
- Microbiological aspects of cheese manufacturing ....................... 7
- Cooling and aging processes of cheddar cheese ......................... 9
- Flavor development during ripening ....................................... 11
- Non-uniform cooling as a process factor in cheese ripening .......... 15

## III. TEMPERATURE EFFECTS ON THE DEVELOPMENT OF CHEDDAR CHEESE FLAVOR AND AROMA

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

## ABSTRACT

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

## INTRODUCTION

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
</tr>
</tbody>
</table>

## MATERIALS AND METHODS

- Experimental design .......................................................... 24
- Descriptive panel ............................................................. 24
- Statistical analysis ......................................................... 25

## RESULTS AND DISCUSSION

- Commercial cheese evaluation ............................................. 27
- Temperature by age interaction effects ................................. 27
- Overall intensity of flavor and aroma .................................. 27
- Sour taste ............................................................................. 29
- Bitter taste .......................................................................... 30
- Sulfur aroma and flavor ....................................................... 32
- Goaty and dirty flavor and aroma ......................................... 33
- Pungent acidic aroma ......................................................... 34
- Fruity aroma and flavor ...................................................... 34
- Salty taste ............................................................................ 35
- Sweet taste ........................................................................... 35
- Buttery aroma and flavor ...................................................... 36
- Nutty aroma and flavor ....................................................... 37
- Yeasty aroma ........................................................................ 37
- Panelist effects .................................................................... 37

## CONCLUSIONS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
</tr>
</tbody>
</table>

## IV. MODELLING OF TIME-TEMPERATURE EFFECTS ON BACTERIAL POPULATIONS DURING COOLING OF CHEDDAR CHEESE BLOCKS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
</tr>
</tbody>
</table>

## ABSTRACT

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
</tr>
</tbody>
</table>

## INTRODUCTION

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
</tr>
</tbody>
</table>

## MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure I-1. Arrangement of 18 kg (40 lb) Cheddar cheese blocks on a pallet in preparation for cooling and aging. ....................... 4

Figure I-2. Cooling of 18 kg (40 lb) Cheddar cheese blocks in the cooling room on a pallet. a. center of the pallet, b. halfway within the pallet, c. outer layer of the pallet. ....................... 5

Figure I-3. Temperature at outside (A) to inside (a) positions in a 290 kg stirred-curd Cheddar cheese block during cooling at 2.2°C ambient temperature (Reinbold et al., 1992). ....................... 6

Figure II-1. Relationship between extent of acid production up to the draining stage, basic structure of the cheese, and production of flavor (Lawrence et al., 1984). ....................... 18

Figure II-2. Proposed ranges in salt in moisture (S/M), moisture in nonfat substance (MNFS), fat in dry matter (FDM), and pH for Cheddar cheese 14 d after manufacture (Lawrence et al., 1984). ....................... 19

Figure II-3. Temperature at center (a,Y or M,J), intermediate (O,S or Q) and surface (A or b) in 290-kg stirred-curd Cheddar cheese blocks during cooling at 2.2°C (Reinbold et al., 1992b). ....................... 20

Figure III-1. Mean flavor intensity scores as a function of time and temperature: overall aroma intensity ....................... 41

Figure III-2. Mean flavor intensity scores as a function of time and temperature: overall flavor intensity ....................... 42

Figure III-3. Mean flavor intensity scores as a function of time and temperature: bitterness ....................... 43

Figure III-4. Mean flavor intensity scores as a function of time and temperature: sulfur flavor ....................... 44

Figure III-5. Mean flavor intensity scores as a function of time and temperature: goaty flavor ....................... 45

Figure III-6. Mean flavor intensity scores as a function of time and temperature: dirty flavor ....................... 46

Figure III-7. Mean flavor intensity scores as a function of time and temperature: pungent acidic aroma ....................... 47

Figure III-8. Mean flavor intensity scores as a function of time and temperature: buttery flavor ....................... 48

Figure IV-1. Temperature distribution in a pallet holding 18.2 kg (40 lb) Cheddar cheese blocks during early aging (Bailey, 1988). ....................... 70

Figure IV-2. Non-starter bacterial counts on Cheddar cheese samples stored at 15-35°C. Symbols correspond to different batches. Lines are drawn using Eq.(1) with parameters listed in Table IV-5. ....................... 71

Figure IV-3. Non-starter bacterial counts for Cheddar cheese samples stored at 12°C. Symbols correspond to different experimental batches. ....................... 72
Figure IV-4. Estimated non-starter bacterial counts at center (A), intermediate (B) and surface (C) locations in 290 kg (640 lb) Cheddar cheese blocks under commercial cooling conditions (Reinbold and Ernstrom, 1985). ........................................ 73

Figure IV-5. Estimated non-starter counts in the center of 18.2 kg palletized Cheddar cheese blocks cooled as shown in Figure IV-1 and for individually cooled blocks calculated as described by Almonacid et al. (1992a,c). ......................... 74
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-1</td>
<td>Descriptors for Cheddar cheese characteristics</td>
<td>49</td>
</tr>
<tr>
<td>III-2</td>
<td>Aroma reference standards for Cheddar cheese</td>
<td>50</td>
</tr>
<tr>
<td>III-3</td>
<td>Aroma and flavor intensity standards for Cheddar cheese</td>
<td>51</td>
</tr>
<tr>
<td>III-4</td>
<td>Mean intensity ratings (0 = none, 15 = extreme) for aroma and flavor</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>characteristics of three samples of commercial medium-sharp Cheddar</td>
<td></td>
</tr>
<tr>
<td>III-5</td>
<td>$F$ statistic significance levels for Cheddar cheese descriptors</td>
<td>53</td>
</tr>
<tr>
<td>III-6</td>
<td>Interaction of temperature and age on overall aroma intensity</td>
<td>54</td>
</tr>
<tr>
<td>III-7</td>
<td>Mean sour and salty intensity scores for the effect of temperature and</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>age on test Cheddar cheese</td>
<td></td>
</tr>
<tr>
<td>IV-1</td>
<td>Initial composition of Cheddar cheese samples</td>
<td>75</td>
</tr>
<tr>
<td>IV-2</td>
<td>Starter (SB) and non-starter (NSB) bacterial counts (CFU/g) for Cheddar</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>cheese samples stored at constant temperature</td>
<td></td>
</tr>
<tr>
<td>IV-3</td>
<td>Kinetic parameters for the growth of non-starter lactic acid bacteria</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>growth during Cheddar cheese aging</td>
<td></td>
</tr>
<tr>
<td>IV-4</td>
<td>Specific growth rate ($d^{-1}$) for the growth of non-starter lactic acid</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>bacteria in a liquid model system</td>
<td></td>
</tr>
<tr>
<td>IV-5</td>
<td>Kinetic parameters for the growth of non-starter lactic acid bacteria</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>growth during Cheddar cheese aging based on an analysis of the entire</td>
<td></td>
</tr>
<tr>
<td></td>
<td>growth curve</td>
<td></td>
</tr>
</tbody>
</table>
CONTRIBUTION OF AUTHORS

In Chapter IV of this thesis (Modelling of time-temperature effects on bacterial populations during cooling of Cheddar cheese blocks), author Kirby was responsible for microbial enumeration methods and related experimental data presented. Graduate student assistants Jorge Bouzas, Ricardo Simpson and Silvana Rocagliolo were responsible for chemical analysis, engineering and computer programming, respectively. All other works was planned, executed and analyzed by author Kirby.
Statistics of the USDA indicate that cheese consumption in the United States is increasing at a projected annual rate of 8.5% and that Cheddar cheese is still the most popular variety consumed in the U.S. (Anonymous, 1985). Consumers prefer a slightly more aged cheese and a premium price is available to manufacturers for sharper cheeses. Thus, incentive exists to manufacture young cheese of the highest quality which can age well, and produce subsequently a Cheddar cheese of balanced flavor and texture.

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 Ernstom, 1988). Unfortunately, there is still the problem of wide variation in sensory properties of Cheddar cheese. 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 remains 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, pressed under vacuum and then cooled in an aging room at 2 to 5°C. As noted in recent publications (Reinbold and Ernstrom, 1988; Reinbold et al., 1992a,b) large differences exist between center and side temperatures which become larger when the cooling temperature is lowered during the early stages of ripening (Figure I-3).

The elevated temperature of the cheese in the early stages of cooling promotes an undesirable, rapid metabolism of lactose, which too often can lead to an 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 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 defects through improved cooling uniformity of Cheddar cheese blocks. On the basis of this goal the specific objectives for this research project were:

1. Identify the principal sensory characteristics of commercial Cheddar cheese of medium sharpness and high acceptability.
2. Study the time-temperature relationships of the sensory characteristics of Cheddar cheese during aging at constant temperature.

3. Study the time-temperature related growth characteristics of starter and non-starter bacteria during aging.
Figure I-1. Arrangement of 18 kg (40 lb) Cheddar cheese blocks on a pallet in preparation for cooling and aging.
Figure I-2. Cooling of 18 kg (40 lb) Cheddar cheese blocks in the cooling room on a pallet. a. center of the pallet, b. halfway within the pallet, c. outer layer of the pallet.
Figure I-3. Temperature at outside (A) to inside (a) positions in a 290 kg stirred-curd Cheddar cheese block during cooling at 2.2°C ambient temperature (Reinbold et al., 1992).
II. LITERATURE REVIEW

Cheddar cheese technology

Cheesemaking is a relatively simple matter based on the removal of moisture from a rennet coagulum. Three major factors involved are extent of acid production, proportion of fat in the curd, and scalding temperature. Given that the latter two manufacturing variables are standardized, the most important variable, by far, in producing cheese of uniform quality is the extent of acid production in the vats (Figure II-1). It is the pH of the cheese, regulated through acid production, which determines the final structure of the curds, and eventually the flavor profile of the ripened cheese (Lawrence et al., 1984). Nevertheless, Cheddar cheese is a difficult variety to manufacture because the long aging period necessary to develop mature Cheddar flavor can be conducive to the formation of off-flavors.

Microbiological aspects of cheese manufacturing

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 adding the starter bacteria to establish the required microbiological population in the milk. The extent of the heating is determined by the manufacturer and is based on preference for a balance of the types of bacteria and enzymes desired in the finished curds (Law et al., 1990). The microflora of heat treated milk used for cheese manufacturing 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 *Bacillus* and *Clostridium* species, staphylococci, coliforms, and lactic acid bacteria such as lactobacilli, pediococci, and leuconostocs. Added to this flora are the starter culture bacteria, usually *Lactococcus lactis* subsp. *cremoris* (*Lc. cremoris*) (Reiter et al., 1967; Cromie et al., 1987; Bhowmik and Marth, 1990).

The milk is then cooled to approximately 30°C which induces growth of the starter bacteria. Acid production by the starter proceeds to the point at which coagulation by the rennet is optimized. 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 formation of the desired texture (Chapman and Sharpe, 1981; Lawrence and Gilles, 1987). The temperature is kept at approximately 30°C to facilitate rennet coagulation.

*Lc. cremoris* is the starter of choice for Cheddar cheese because it has the advantages of producing good cheese flavor, being free from off-flavor production, and developing a steady rate of acid production from lactose metabolism (Chapman and Sharpe, 1981). Starter bacteria become physically entrapped in curd during coagulation and are drained off in fewer numbers in the whey. Due to this distribution, lactic acid build-up is disproportionately higher in the curd than in the whey (Chapman and Sharpe, 1981).

Once the coagulum has formed to the desired firmness, the curd is cut, stirred, and scalded. Scalding is necessary to shrink the protein matrix and expel the whey from the curds, while maintaining a viable starter culture. Inhibition and destruction of a typical starter culture for Cheddar cheese begins at 40°C (Sandine, 1989). As the curds whey off after cutting, they are piled and repiled to develop the characteristic
texture of Cheddar. This process insulates the curds, maintaining the temperature in the upper 30°C range. This further promotes the growth of starter and concurrent acid production.

In general, Cheddar cheese is produced under standard manufacturing conditions, but each manufacturer induces special conditions which impart a unique character to the microflora. The dairy environment can become the permanent habitat of variable strains of adventitious organisms (Khalid and Marth, 1990). Resident flora inoculates each batch of cheese such that a particular flora can become signature for a plant, imparting individual flavor overtones to cheese made in that plant (Chapman and Sharpe, 1981).

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

Given normal acid production, good milk quality and proper sanitation, Cheddar cheese will begin the ripening process with a chemical composition that falls within the inner square of Figure II-2 and will result in an acceptable product (Lawrence et al., 1984).

**Cooling and aging processes of cheddar cheese**

"Green" or young cheese is composed of casein proteins, fat, moisture, salt, lactose, lactic acid, whey proteins, and minerals. During the ripening process, raw curd evolves from a rubbery, bland mass to a firmer, more brittle cheese with a strong
Cheddar flavor (Barlow et al., 1989). Ripening is a process of enzymatic digestion of milk components to induce chemical changes within the cheese and includes glycolysis, proteolysis, and lipolysis. Enzyme sources include the starter culture, non-starter bacteria, rennet, and endogenous milk enzymes (Chapman and Sharpe, 1981).

The rate of ripening is controlled by the composition of the cheese, most especially the pH, moisture and salt levels, and the temperature of the cheese. These factors all influence the microflora which develops in the cheese. The ripening process may take months to years depending on the degree of "sharpness" desired in the end product. The temperature of storage is generally from 4-15°C (Cromie et al., 1987).

Starter bacteria die out during the ripening process as do most other bacteria including most enterococci and the leuconostocs (Bautista and Kroll, 1988; Khalid and Marth, 1990). Maximum levels of starter bacteria, $10^8$ CFU/g for Lc. cremoris, are reached at the milling stage. Lc. cremoris is inhibited by 2% NaCl, thus numbers begin to decline after salting (Chapman and Sharpe, 1981).

Most unwanted bacteria are suppressed by the salt and pH levels found in Cheddar cheese except for Escherichia coli which requires almost 12% NaCl for inhibition and may actually be stimulated by 3% (Bautista and Kroll, 1988). Coliforms tolerate acid, salt, are not inhibited by starter bacteria, ferment lactose, and grow at temperatures typical in cheesemaking. Control through sanitation is essential to keep numbers low and prevent excessive growth and subsequent flavor and body defects in the early stages of ripening (Dommett, 1970).
Leuconostocs and micrococci may be present in small numbers, but die out or do not multiply in the ripening cheese (Chapman and Sharpe, 1981). Only the non-starter lactic acid bacteria, including lactobacilli and pediococci, increase in numbers during ripening (Reiter et al., 1967; Thomas et al., 1985).

*Lactobacillus* species persist and are the dominant organisms during ripening (Khalid and Marth, 1990). They reach counts of approximately $10^1$-$10^4$ CFU/g in the curd, reaching counts of $10^6$-$10^8$ CFU/g in 10-60 d in the ripening cheese (Peterson and Marshall, 1990). Since the starter bacteria are faster growing organisms, the numbers of lactobacilli remain low until such time as the greater acid tolerance of the lactobacilli allow these organisms to out-compete others. These maximum levels are maintained, then decline after 4-6 months under standard ripening conditions. Species often include *Lb. casei, Lb. plantarum, Lb. brevis, Lb. buchneri* (Chapman and Sharpe, 1981).

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, CO$_2$, and formic acid (Kandler and Weiss, 1986).

**Flavor development during ripening**

The component balance theory for Cheddar cheese proposes that characteristic Cheddar flavor is the result of a synergistic blend of components which must be brought together in the proper proportion for desirable overall characteristics. This theory has been accepted by researchers in spite of the disagreement that continues on
the relative contribution of the various components (Aston and Dulley, 1982; Barlow et al., 1989; Vandeweghe and Reineccius, 1990). The precise nature of the reactions which produce flavor compounds and the way in which their relative rates are controlled is not clearly understood. This is due, in part, to deficiency in knowledge about the compounds which impart typical flavor to Cheddar cheese, and, also, to the complexity of the cheese microflora as potential producers of flavor compounds (Lawrence and Gilles, 1987).

A number of flavor characteristics are associated with the microbiological flora of the cheese. This has been established by cheeses made with delta-glucono-lactone instead of starter bacteria under aseptic conditions which do not develop a typical Cheddar flavor (Reiter et al., 1967; McGugan et al., 1968).

Starter lactococci are not directly responsible for the development of characteristic Cheddar flavor compounds. These species are homolactic under the conditions of cheese manufacture, producing mostly lactic acid, some volatile acids, ethanol, lactate salts and only small quantities of acetate and diacetyl from lactose fermentation (Marshall and Law, 1984; Thomas et al., 1979; Barlow et al., 1989).

Lactic acid bacteria contribute to the production of flavor and aroma compounds in Cheddar cheese in three ways: 1) in the production of trace aroma compounds, 2) by the action of primarily intracellular proteases and lipases liberated during autolysis of dead cells, and 3) via the conditions of low pH and negative redox potential developed which allow for the chemical production of reduced sulfur compounds as chief constituents of Cheddar flavor (Law and Sharpe, 1978).
Lactose fermentation by lactic acid bacteria constitutes the basis of glycolytic reactions during the ripening process. 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. Rapid cooling to 10°C limits the rate and extent of growth by heterofermentative non-starter organisms and thus their fermentation products (Fryer, 1982).

Except for pediococci, secondary lactic-acid bacteria (non-starter) tend to metabolize lactose by heterofermentative pathways. Fermentation end products include mostly acetate, formate, and ethanol with as little as 1% conversion to lactate (Thomas et al., 1979). When such growth is extensive, cheese quality is often less than satisfactory because of fermented and sour off-flavors (Fryer, 1982; Lawrence et al., 1984).

Many of the components of Cheddar flavor develop through enzymatic action. Starter and non-starter bacteria are sources of some of these enzymes (Moskowitz, 1980). However, bacterial enzymes released by lysis of dead starter cells do not in themselves produce flavor compounds except for a few low molecular weight precursors to aroma compounds (e.g. cysteine and methionine) (Law et al., 1976).

Proteolysis is important during cheese ripening in part because it releases short chain peptides and amino acids which contribute to flavor development. It occurs through action of cheese microflora, coagulants, and natural milk proteases (Fox, 1989; Bhowmik and Marth, 1990). Proteolysis is one of the essential roles of bacterial enzymes during ripening and has been found to be controlled largely through ripening
temperature (Lawrence et al., 1984). These enzymes have optimum activity under neutral to alkaline pH conditions, but retain some activity under the acidic conditions found in ripening Cheddar (Cliffe and Law, 1979).

Starter proteases and peptidases convert the large peptides to smaller peptides and free amino acids. Amino acids may be further reduced by microorganisms to ammonia and organic acids or oxidized to CO₂ and amines. Non-starter bacteria contribute to a greater diversity of small peptides and free amino acids (Retter et al., 1969; O'Keefe et al., 1976).

Small chain peptides derived from the cleavage of α₅-casein tend to be strongly hydrophobic. The formation of these peptides accounts for the bitter flavor that develops during the aging of cheese (Lindsay, 1985). Manning (1978) found that bitterness reached a maximum intensity at 2-4 months when ripened at 13-14°C, but declined rapidly to a minimum at 10 months. In comparison studies of low and traditional ripening temperatures, Law et al. (1979) concluded that bitterness was more marked at low ripening temperatures (6°C) possibly because the peptidases necessary to hydrolyze bitter peptides required higher temperatures (13°C) for adequate activity. Studies comparing cheeses made with and without adventitious bacteria show that more intense Cheddar flavor of higher quality was achieved in cheeses where adventitious bacteria were present. Fewer flavor defects, in particular bitter flavor, were detected in cheese with adventitious bacteria present (Law et al., 1979).

In Cheddar cheese, lipolysis results through action of lipases from the starter culture, the secondary flora, and naturally occurring milk lipases (Stadhouder and Veringa, 1973; Bhowmik and Marth, 1990). Fat hydrolysis products of the greatest
importance are the volatile lower shorter chain fatty acids, especially butyric, caproic, caprylic, and capric (Lamparsky and Klimes, 1981).

Some short chain fatty acids are produced by lactic acid bacteria during ripening. Butyric acid is the most plentiful acid produced in this way in Cheddar cheese (Stadhouder and Veringa, 1973). Other fatty acids, particularly those greater than C4, may not be produced from lipids. Stadhouder and Veringa (1973) suggested that the glycerol ester hydrolases of lactic acid bacteria hydrolyze triglycerides in cooperation with milk and Gram negative rod lipases.

Some lactic acid bacteria produce an esterase capable of esterifying butyrate and hexanoate to produce the corresponding ethyl esters (Hosono et al., 1974). Ethyl butyrate and ethyl hexanoate have been found to be responsible for the fruity flavor defect in Cheddar cheese (Bills et al., 1965).

**Non-uniform cooling as a process factor in cheese ripening**

A significant portion of commercial Cheddar cheese is criticized for off-flavor defects (Bodyfelt, 1986; Bodyfelt et al., 1988). Studies of the effect of ripening temperature on the flavor of Cheddar cheese have shown cooling after pressing to be the most important single factor (Law et al., 1979). Even with good manufacturing practices, Cheddar cheese may develop off-flavors as a result of failure to control the temperature at which the cheese matures, particularly in the first few days of ripening (Lawrence and Gilles, 1980). Law et al. (1979) showed that the difference between low (6°C) and "traditional" (13°C) ripening temperature effects on flavor intensity was greater than either the presence of non-starter lactic acid bacteria or the type of starter
culture used. An early survey of several cheese plants in the United States indicated that the measure of control of post hoop curd handling did not approach standardization. Manufacturers polled indicated that the time to cool pressed wrapped cheese blocks to ripening temperature ranged from 24 to 28 hours and 54% expressed they lacked control over day-to-day variations in post hoop handling (Vedamuthu et al., 1969). More recently, researchers found that the temperature in the center of a 290-kg block actually rose within the first 24 h of cooling by about 4°C (Reinbold et al., 1992a,b). After 24 h of cooling, center temperature decreased by only 2°C. Thereafter, it took 12 to 15 d to cool the center from an initial temperature of 32°C to the cooling room temperature of 2.2°C (Figure 11-3).

The rate of cooling of cheese in the first days of aging is the most important factor in controlling cheese microflora, particularly the non-starter bacteria (Fryer, 1982; Lawrence et al., 1984). However, an initial temperature of 22°C or higher is not unusual for cheese that is placed immediately after pressing in bulk bins, a common commercial practice (Lawrence et al., 1984; Reinbold et al., 1992a,b). Stacking of blocks on pallets and stacking of pallets on each other influences the heat transfer out of the cheese blocks and, thus, the rate of ripening. This difference in initial cooling rate can result in different ripening rates between blocks and within blocks. Ripening is also affected by the size of the block to the extent that there is non-uniformity of cooling throughout the block.

Research has shown widely varying growth rates of non-starter bacteria at different constant cooling/ripening temperatures (Turner and Thomas, 1980). Miah et al. (1974) proposed that controlling of cooling temperature offers the best means of
controlling cheese flavor through controlled bacterial growth. Given standardized manufacturing conditions, if starter and non-starter growth are controlled through rapid cooling so as not to reach concentrations that give discernible off-flavors, the flavor that develops in Cheddar cheese is likely to be acceptable to most consumers (Lawrence et al., 1984).
Figure II-1. Relationship between extent of acid production up to the draining stage, basic structure of the cheese, and production of flavor (Lawrence et al., 1984).
Figure II-2. Proposed ranges in salt in moisture (S/M), moisture in nonfat substance (MNFS), fat in dry matter (FDM), and pH for Cheddar cheese 14 d after manufacture (Lawrence et al., 1984).
Figure II-3. Temperature at center (a, Y or M, J), intermediate (O, S or Q) and surface (A or b) in 290-kg stirred-curd Cheddar cheese blocks during cooling at 2.2°C (Reinbold et al., 1992b).
III. TEMPERATURE EFFECTS ON THE DEVELOPMENT OF CHEDDAR CHEESE FLAVOR AND AROMA

ABSTRACT

Cooling of freshly formed Cheddar cheese is believed to be one of the processing steps which requires tighter control to achieve more uniform and consistent product quality. Cheese samples, obtained after pressing, were rapidly cooled to 5, 15, 25, and 35°C. Commercial samples and test cheese at 7, 30, 60, 90, and 120 d of ripening were evaluated by a trained descriptive panel. Most sensory characteristics of experimental cheese increased in intensity as a function of the interaction of time and temperature. The perception of sour and salty taste were affected by temperature, but at equal rates over time. Buttery aroma and flavor tended to decrease in intensity as a function of time and temperature.
INTRODUCTION

Cheese ripening has been defined as the controlled decomposition of a rennet coagulum of milk constituents (Lawrence and Gilles, 1987). As this process occurs a balance of taste and aroma (flavor) compounds is formed. The nature of this process is influenced in part by the microflora of the cheese. Controlled activity of cheese microflora is an important factor in prevention of off-flavor and aroma development (Fryer, 1982; Lawrence et al., 1983). Conditions which favor the die-off of starter bacteria (Lactococcus lactis subsp. cremoris) prior to complete lactose utilization open the way for formation of off-flavors such as sour. Heterofermentative metabolism of lactose by non-starter bacteria produce as by-products formic acid, ethanol, and acetic acid (Law, 1984). Excesses of these compounds can impair the flavor balance of Cheddar cheese (Moskowitz, 1980). Rapid cooling of cheese blocks to ripening temperatures is the primary means to control the microflora activity and thus promotes homofermentative metabolism (Fryer, 1982). Law et al. (1979) found that ripening temperature was the most important single factor effecting Cheddar flavor intensity. Their data suggest that higher ripening temperature promotes faster flavor development.

Conochie and Sutherland (1965) found a correlation between occurrence of cheese flavor defects and uneven cooling of blocks of Cheddar which have been stacked closely on pallets. After pressing, these blocks are still warm and are insulated to the inside of the pallet. In a survey of Cheddar cheese manufacturers conducted in 1969 it was concluded that lack of control in the cooling step from pressing to curing
was responsible for considerable variation within lots (Vedamuthu et al., 1969). This can lead to various off-flavor defects. Experienced cheese graders have been reported to criticize 30 to 40% of all American Cheddar cheese as being high acid (sour) and bitter (aged cheese) in off-flavor (Bodyfelt, 1986; Bodyfelt et al., 1985). Miah et al. (1974) studied the effects of four pressing and 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 ripening temperature (6 and 13°C) on flavor preference. They concluded that higher temperature ripening for longer periods was associated with lower preference scores. Production of strong off-flavors was cited as the cause of low preference scores compared to a standard.

The temperature of the post-hoop cheese block ranges between a high of 35°C at pressing to the temperature at ripening (3.5 to 12°C). During the cooling period, a temperature gradient is established within the block of cheese (Reinbold and Ernstrom, 1985). The rate of cooling of any given point within a cheese block will depend, in part, on the temperature profile over time at that point. The effect on sensory characteristics will be some function of the combined effects at the various temperatures within the profile. The purpose of this study was to define and quantify the effect on sensory characteristics of constant cooling temperatures as they varied over the general range of processing temperatures (i.e., 5 to 35°C).
MATERIALS AND METHODS

Experimental design

Eighteen kg (40 lb) Cheddar cheese blocks produced from flash-heated milk by a large regional processor (Tillamook County Creamery Assoc., Tillamook, OR) were cut directly after pressing into pieces measuring 6.4 cm x 8.9 cm x 14.6 cm (2.5" x 3.5" x 5.75"). Each piece was vacuum shrink-wrapped in commercial O₂-barrier cheese film. Samples were placed into incubators at 5, 15, 25, and 35°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 7, 30, 60, 90, and 120 d, except 35°C cheese samples which were discontinued from testing after 60 d.

The first two of the six batches of cheese sampled were used for training and preliminary investigation. The four batches used for testing were collected one week apart during June, 1989. The batch #4 cheese stored at 35°C was not tested at 60 d due to the development of intense off-odors which hindered proper evaluation of the samples.

Descriptive panel

A descriptive panel of nine individuals was selected for participation in the project. Panelists developed a list of descriptors to characterize a variety of commercial and experimental samples. These samples included commercial and preliminary test samples selected to represent the range of sensory characteristics anticipated in the study (Table III-1). Reference standards were selected for most of
the descriptors (Table III.2). A 16-point scale anchored with intensity descriptors (0 = none, 15 = extreme) was used to rate the intensity of the characteristics. Intensity reference standards were used to reduce variability among panelists (Table III-3, Meilgaard et al., 1987). A ballot was developed to use in training the panel and later to test the cheese.

Blind-coded test samples from each storage temperature were served in random order to panelists in isolated sensory booths. Red lights were employed to mask appearance differences among cheese samples. Cheese blocks were tempered to 10 to 12.8°C before serving. A cheese core trier was used to obtain, immediately prior to serving, samples (1.3 cm diameter by 6.4 cm length) free of surface oxidation artifacts. Servings were placed in covered plastic containers for immediate presentation to panelists.

Statistical analysis

Data were analyzed through analysis of variance techniques with a compound \( F \) test as described by Schultz (1955). Temperature, age, batch, and panelist were established as the main effects. Temperature and age were treated as fixed effects. Batch and panelist effects, since they were viewed as a representative sampling from a population, were treated as random effects (Lundahl and McDaniel, 1988). Estimates of mean square values were generated using the General Linear Models procedure for unbalanced data on the SAS statistical software package (Version 6.02, SAS Inst. Inc., Cary, NC) on an IBM system 4381 (IBM Corp., Boca Raton). The objective was to study the effects of temperature and age concurrently. For that reason, all descriptors
were first tested for the interaction of age and temperature using the GLM procedure for unbalanced data on the entire data set. For those descriptors which were found to be insignificant (P > 0.05) for the interaction effect of temperature and age, a second test was run for the effects of age and temperature alone. In these cases, the mean square values were generated on data collected at all storage temperatures through 60 d of testing only. This was done to prevent introduction of a bias from 90 and 120 day measurements.

Due to the biased nature of the missing data point for batch #4, 35°C, 60 d, a synthetically-derived, biased estimate of the treatment by age mean for 35°C at 60 d was required. This was accomplished by calculation of an estimate of the error terms associated with values for complete blocks of batch by temperature means. These values were then substituted back into the model equation for point estimation of the missing value (batch #4, 35°C). This value was then used to calculate the corrected mean value at 35°C and 60 d (L. Calvin, Personal Communication, Dept. of Statistics, OSU). These values are used in this analysis where temperature, age, and temperature by age means are reported.

LSD values were used to determine differences between the means where significance (P < 0.05) was established by the type 1 compound F test for temperature, age, and treatment by age effects. These values were calculated using a type 2 F test and a t-value at 5%.
RESULTS AND DISCUSSION

Commercial cheese evaluation

During the later stages of training, the panel was asked to evaluate samples of commercial medium sharp cheese. These samples were manufactured by the same supplier as were the test cheese (Table III-4). A formal study of the characteristics of commercial Cheddar is not consistent with the objectives of this study. However, the values shown here have been helpful in the relative evaluation of the test cheese to follow.

Temperature by age interaction effects

All aroma and flavor characteristics were significant for temperature by age interaction except nutty aroma, and nutty, sour, and salty flavor (Table III-5). Nutty aroma showed no significance at less than 5% for either treatment effect. Nutty, sour, and salty flavor were significant for the effects of both age and temperature.

All characteristics which showed significance due to some treatment effect generally showed increasing intensity with increasing age, temperature, and age by temperature. The exception to this was buttery aroma and flavor which generally decreased with increasing age by temperature interaction.

Overall intensity of flavor and aroma

Initial testing for the significance of the interaction of temperature and age has the advantage of revealing the relationship between any two conditions of temperature and age. This is not the case when the temperature or age effect are tested
independently. Thus the latter was used only when the interaction effect was insignificant. Table III-6 gives temperature by age statistics for overall aroma intensity. These means are represented graphically in Figure III-1. At the first test period (7 d), the difference in the means for overall aroma intensity was significantly higher at successively higher temperatures. This trend continued through 120 d. The cheese stored at 5°C increased only slightly in overall aroma intensity score from 7 to 120 d. The cheese aged at 15°C followed a similar pattern, but at higher average intensity scores and with a slightly greater overall increase in intensity. The cheese aged at 25°C increased in overall intensity through 90 d, but showed no significant change after that. The cheese aged at 35°C increased in overall intensity through 30 d, but showed no significant change between 30 and 60 d.

In general, overall flavor intensity followed the same patterns as did the overall aroma intensity, but to a greater degree of both intensity and rate of increase (Figure III-2). Flavor intensity was perceived at slightly lower levels at the first test period, but generally increased at greater rates throughout ripening. At the last test period (120 d), cheese was higher in flavor intensity than aroma intensity. Again, the cheese stored at 35°C showed less increase in flavor intensity between 30 and 60 d than between 7 and 30 d.

The commercial samples evaluated during training (Table III-4) were found to have a range of aroma and flavor intensity equivalent to experimental samples ripened between 5 and 15°C for 60 d. This is consistent with the actual manufacturing practices of the processor; pallets of 18 kg cheese blocks are ripened in a room at 3.3 to 4.4°C for a minimum of 60 d. The experimental cheese ripened at 35°C was
significantly higher in overall flavor and aroma intensity at 7 d than the 15°C, 60 day old cheese (i.e., the one similar to commercial cheese samples). This observation adds perspective and emphasis to how rapidly high temperature ripening affects cheese flavor characteristics. Aston et al. (1985) also found total flavor to be a function of time and temperature. Their findings corroborate the conclusion of Law and Sharpe (1978) that maturation temperature is the most important factor in determining flavor intensity.

A recent review (Lawrence and Gilles, 1987) of the role of starter bacteria concluded that enzymes from both viable and non-viable starter cells are contributing factors in flavor development in Cheddar cheese. Various studies of the contribution of non-starter bacteria to flavor development have shown that cheese made in open vats develops an overall intensity of Cheddar flavor more rapidly than experimental cheese containing populations of exclusively starter culture microorganisms (Reiter et al., 1967). In addition, the densities of non-starter bacteria are quite important (Lawrence et al., 1983), particularly in the early stages of ripening. The experimental cheese in this study exhibited increasing growth rate of non-starter bacteria as a function of temperature. This correlates to the observations of the panel concerning the increase in overall aroma and flavor intensity with increasing time and temperature.

**Sour taste**

The interaction of temperature and age on sour flavor was not significant. However, the F value for differences in the effect of temperature only at 7, 30, and 60 d showed significance. Sourness generally increased with an increase in temperature.
except that no significant difference was found between the average intensity at 15°C and that at 25°C (Table III-7). The F value also showed significance for the difference between age means. Sourness increased only between 7 and 30 d. These results are in general agreement with chemical profiles reported by Bouzas (1991).

The proportion of residual lactose (unmetabolized) is highest in Cheddar curd at the beginning of the cooling process. Starter bacteria are not entirely inhibited by the salting process and this microflora can be expected to continue to metabolize lactose (Lawrence et al., 1984). In addition, non-starter bacteria such as lactobacilli and pediococci affect the rate of metabolism of lactose. Turner and Thomas (1980) found that the temperature of cooling played an important role in the rate of lactose metabolism during aging. These investigators found that at 22°C, residual lactose in Cheddar was utilized more than twice as fast as when cheese was cooled immediately to 12°C. Thus, our observations that perceived sourness was generally a function of temperature (cooling rate), and that increased sourness was most readily noted during the early stages of ripening (when metabolism of lactose was greatest) is consistent with the findings reported by other researchers (Fagen et al., 1952).

More rapid growth of non-starter bacteria encourages heterolactic fermentation of lactose as well (Lawrence et al., 1984). Production of acetic acid in addition to lactic acid through microbiological activity contributes to overall sourness.

**Bitter taste**

At the first test period (7 d), the cheese stored at 35°C was significantly more bitter flavor and the one kept at 5°C was significantly less bitter than the cheese aged
at 15 and 25°C (Figure III-3). The rate of increase was higher with cheese stored successively higher temperature with significant differences in all temperatures at all ages beginning at 30 d. The experimental cheese ripened at 25 and 35°C were significantly higher in bitter flavor at 60 d than commercial cheese which had been ripened for 60 d or longer.

Bitterness in Cheddar cheese has been attributed to the proteolytic breakdown of casein to peptides which manifest a bitter taste. Rennet is largely responsible for hydrolysis of casein to non-bitter, high molecular weight peptides. Starter bacteria peptidases reduce these intermediate products of proteolysis to bitter, low molecular weight peptides (O'Keefe et al., 1976). Intracellular peptidases released during die-off of the starter bacteria are largely responsible for this activity (Lawrence et al., 1978). Accelerated die-off of starter bacteria with increasing ripening temperature can be expected to contribute to the more rapid development of bitter flavor. 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; Lee et al., 1990). Lee et al. (1990) found that several species of lactobacilli added to the starter culture during cheese manufacture resulted in bitterness compared to starter culture that contained only *Lactococcus* species. It is this species specific proteolytic activity which may explain why some researchers report a decrease in bitterness with time while others report an increase (Law and Sharpe, 1978; Barlow et al., 1989).
Sulfur aroma and flavor

Sulfur flavor was perceived to be a very minor component in cheese at 7 d, reaching just detectable levels in the 35°C storage temperature cheese only (Figure III-4). At 30 d, the cheese at 5°C was lower in sulfur-like flavor and the one at 35°C was higher than the ones at 15 and 25°C. At 60 d, the cheese stored at 25 and 35°C were higher than the others. At 90 and 120 d, intensity increased with increasing temperature. The means for pungent sulfur aroma revealed the same trend of development as sulfur flavor but at lower intensity levels. The intensity of pungent sulfur aroma ranged from 0.97 (just detectable) to 4.19 (little more than slight).

Volatile compounds are an essential factor in Cheddar flavor (McGugan et al., 1979). The literature on the contribution of sulfur compounds to Cheddar flavor has been conflicting. Headspace analysis has shown a strong correlation between sulfur-containing volatile (H₂S, methanethiol) and the presence of normal Cheddar aroma (Law et al., 1979; Green and Manning, 1982). However, another study concluded that sulfur compounds are not good indicators of flavor development (Aston and Douglas, 1983).

Starter bacteria have been found to contribute to the sulfur note in Cheddar flavor. The means is through development of the proper conditions of pH and redox potential and the production of precursors to the formation of volatile sulfur compounds (Law et al., 1976). Non-starter bacteria have also been found to contribute to redox potential conditions to varying degrees (Thomas et al., 1985). In this study, sulfur aroma and flavor characteristics tended to increase with increasing age and
temperature of cheese maturation. These findings were consistent with the microbiological studies on experimental Cheddar cheese samples (Chapter IV).

**Goaty and dirty flavor and aroma**

At 7 d, the 35°C storage temperature cheese was higher in "goaty" flavor and the cheese at 5°C was lower in this character than cheese at any other storage temperature but same age (Figure III-5). Cheese ripened at 35°C was already significantly higher in goaty flavor than the commercial cheese tested, which was rated just detectable. Eventually, cheese aged at 25 and 35°C reached a level of goaty flavor that coincided with slight to moderate on our scale. Cheeses stored at 5°C showed no real change in goaty intensity over the total test period with goaty flavor judged to be just detectable. Goaty aroma showed trends similar to goaty flavor but to a lesser degree. Dirty-like aroma and flavor followed trends similar to goaty flavor, but generally with somewhat less intensity (Figure III-6).

Low molecular weight free fatty acids (< C₄) are produced by lactic acid bacteria during ripening (Stadhouder and Veringa, 1973). The most important of these is butyric acid which was the reference compound used by the panelists to describe goaty flavor and aroma. Stadhouder and Veringa (1973) suggested that the longer chain fatty acids (> C₄) were produced through deamination of amino acids (e.g., isovaleric acid from valine). Lamparsky and Klimes (1981) identified isovaleric acid as a constituent of Cheddar flavor. Dilute isovaleric acid was chosen by the panelists as characteristic of dirty flavor and aroma. Puchades et al. (1989) showed that valine was one of the principal amino acids liberated through proteolytic activity of starter
and non-starter bacteria. Increase in temperature from 6 to 15°C intensified proteolysis. Some strains of lactobacilli have been associated with a reduction of valine levels during the latter stages of ripening. The authors suggest that this reduction is associated with reactions occurring in the cheese, resulting in the formation of deamination products (Puchades et al., 1989).

**Pungent acidic aroma**

The development of a pungent acidic aroma followed a similar trend to goaty and dirty characteristics, increasing in intensity as a function of increasing time and temperature (Figure III-7). The panelists associated this aroma with that of dilute formic acid. Fryer (1982) suggested that control of the growth of non-starter lactic acid bacteria, particularly *Lactobacillus*, through rapid cooling to 10°C prevented the heterofermentative metabolism of lactose. This pathway in the early stages of ripening leads to defects associated with excess formic acid (Thomas et al., 1979).

**Fruity aroma and flavor**

"Fruity" flavor was determined at just detectable levels (.66 to 1.44) at 7 d and at all temperatures. The cheese aged at 5°C remained at or below just detectable levels throughout the test period. All other samples increased in intensity as a function of time and temperature, reaching an intensity range of 1.1 to 3.7 for final test periods. Fruity aroma showed similar trends. Commercial samples ranged slightly over the just detectable level which was less than the cheese aged at 35°C for 30 d.
Conochie and Sutherland (1965) found that "fruitiness" is often a function of ripening temperature. Manning (1979) showed a correlation between the fruity flavor characteristic and the ethanol content in the head space. Thomas et al. (1979) associated the production of ethanol with heterofermentative metabolism of lactose by non-starter bacteria in the early stages of ripening at higher temperatures. This study showed that the samples aged at 5°C did not increase significantly in fruitiness during ripening. This finding supports Fryer's (1982) contention that rapid cooling to 10°C prevents heterofermentative activity.

**Salty taste**

The $F$ statistic for the interaction of temperature and age as related to salty taste was not significant. As with sourness, this indicates no difference in the rate of development of a salty flavor characteristic as a function of time and temperature. However, significance was detected in the $F$ value for temperature and age effects alone. The analysis of the means for these treatments indicated a perceived saltiness increasing through the 25°C storage temperature (Table III-7). Saltiness also appeared to increase gradually with age contrary to what was expected.

**Sweet taste**

Sweetness was judged to be a minor component in the experimental cheese. Differences in the intensity of the temperature treatments over time only became apparent at 60 d. The cheese aged at 25 and 35°C was significantly higher in sweetness than the one aged at 5 and 15°C through 60 d. The cheese aged at 25°C
continued this trend through 120 d. In the case of cheese aged at 5 and 15°C there were no substantial changes perceived through 120 d and sweetness scores ranged from 1.31 to 2.14. The cheese aged at 25°C gradually increased through 120 d to a maximum intensity score of 3.42 (slight). This was significantly higher than the 5 and 15°C storage temperature cheese by 60 d. The cheese aged at 35°C increased in sweetness between 30 and 60 d. Changes in perceived sweetness may have been associated with fruitiness perception rather than carbohydrate content, since the latter decreases over time (Lawrence and Gilles, 1987).

**Buttery aroma and flavor**

At 7 d, no difference in buttery flavor was perceived among any of the storage temperatures (Figure III-8). Thereafter, the cheese stored at 25 and 35°C declined in buttery flavor as a function of time and temperature. The cheese stored at 15°C was not perceived as significantly different from the cheese aged at 5°C until the last test period (120 d) when the buttery intensity was slightly less. Generally, buttery aroma followed the same trend and was perceived at similar intensity levels.

In the cheese tested earliest, buttery was perceived as one of the more dominant flavor components. As aging continued the intensity of perception decreased at rates consistent with higher temperature treatment. This phenomenon is perhaps due to 1) a masking effect by other components of increasing intensity, 2) alteration of the compounds responsible for buttery flavor which resulted in loss of perception, or 3) a combination of both of these factors.
Nutty aroma and flavor

$F$ values for the interaction of temperature and age on nutty flavor and aroma were not significant. The $F$ statistic for the effects of age or temperature was significant to 5% only and intensity ratings overall for nutty flavor were less than slight. As expected, nutty aroma was judged to be of only slight intensity throughout the 120 d of ripening time for all temperatures. No significance was found for the effects of storage temperature, age, or temperature by age interaction on nutty aroma. Thus, although present, nuttiness was not perceived as a major factor during ripening. This was generally consistent with the nuttiness level perceived in the commercial cheese samples.

Yeasty aroma

Yeasty aroma was perceived as undetectable by most of the panelists except in the cheese stored at 25 and 35°C after 60 d. In these samples, it was judged to be just detectable.

Panelist effects

The panelist effect was tested and found to be significant for all flavor and aroma characteristics; this implies that panelists were using different parts of the scale to define the given intensity of the perceived characteristic. The $F$ tests for the interaction of panelists by temperature by age were found to be significant for several of the descriptors. These descriptors also showed significance for temperature by age interaction (Table III-5). Descriptors which were found to be significant for the effects
of temperature or age alone were also found to be significant for the interactions of temperature by panelist and age by panelist (Table III-5).

Although panelist by treatment interaction was present for several of the descriptors, usually individual panelists agreed with the general trends as indicated by average values. With certain descriptors (overall aroma intensity, dirty aroma, sour, bitter, goaty, dirty, and sulfur flavors), significance of the interaction effect appeared to be related to how an individual panelist perceived the relative magnitude of the intensity on the 16-point scale. For example, one panelist may have perceived that the rate of increase of intensity with storage temperature over time was greater or less than the rate perceived by other panelists. This means that various panelists were using differences between parts of the scale in relatively different ways.

With three of the flavor descriptors (salty, sweet, fruity) confusion appeared to exist among panelists about the relative perception of these characteristics. Some panelists described increasing intensity with increasing temperature over time, others described decreasing intensity or no change. In describing intensity of yeasty aroma, some panelists consistently scored this characteristic as not present in all or most of the samples. This may have indicated below threshold levels or panelist confusion in identifying this particular characteristic.

CONCLUSIONS

The characteristics analyzed in this study represent an extensive description and quantification of the aroma and flavor profile of experimental Cheddar cheese by a trained sensory panel. In general, flavor and aroma intensities of the experimental
cheese increased as a function of time and temperature; exceptions to this were the nutty and buttery aroma and flavor characteristics. Many of these sensory characteristics were closely associated with microbiological changes which were also correlated to time and storage temperature.

Mean intensity scores for overall aroma and flavor intensity reflected the changing character of the cheese early in the ripening phase. Flavor and aroma intensity was described by the panelists as being in the moderate range, and increasing with the given storage temperature. The main components (more than slight) of the flavor at 7 d were sour, salty, and buttery flavor and aroma. Secondary characteristics (slight) included goaty flavor and aroma, pungent acidic aroma, nutty flavor and aroma, and bitter flavor. All others tested were judged to be at threshold levels with the exception of yeasty which was not detected by most panelists.

As overall intensity increased with time, so did bitter flavor, goaty aroma and flavor, dirty aroma and flavor, pungent acidic aroma, sulfur flavor and aroma, and fruity aroma and flavor. These trends reflected the growing complexity of the cheese character with time and temperature. These components reached or exceeded levels consistent with the initially main components of sour, salty, and buttery aroma and flavor. The latter tended to stabilize or decrease in the presence of the other components. This study reflects the importance of temperature on the desirable characteristics of Cheddar cheese aroma and flavor. Several components (bitter, goaty, dirty, fruity) are integral to the overall character of Cheddar cheese as reflected by evaluations of commercial samples evaluated in this study. However, excessive levels prompted complaints by the panelists about the disagreeable character of some of the
test cheese. Sensory evaluations at day 7, indicate that cheese stored at high temperatures quickly reach intensity levels for these components unsuitable for commercial Cheddar cheese.
Figure III-1. Mean flavor intensity scores as a function of time and temperature: overall aroma intensity
Figure III-2. Mean flavor intensity scores as a function of time and temperature: overall flavor intensity
Figure III-3. Mean flavor intensity scores as a function of time and temperature: bitterness
Figure III-4. Mean flavor intensity scores as a function of time and temperature: sulfur flavor
Figure III-5. Mean flavor intensity scores as a function of time and temperature: goaty flavor
Figure III-6. Mean flavor intensity scores as a function of time and temperature: dirty flavor
Figure III-7. Mean flavor intensity scores as a function of time and temperature: pungent acidic aroma
Figure III-8. Mean flavor intensity scores as a function of time and temperature: buttery flavor
TABLE III-1. Descriptors for Cheddar cheese characteristics.

<table>
<thead>
<tr>
<th>Aroma:</th>
<th>Overall Intensity, Buttery, Nutty, Fruity, Pungent Acidic, Pungent, Sulfur, Goaty, Dirty, and Yeasty.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor:</td>
<td>Overall Intensity, Sour, Salty, Bitter, Sweet, Buttery, Nutty, Fruity, Goaty, Dirty, and Sulfur.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aroma Characteristic</th>
<th>Reference Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutty</td>
<td>lightly roasted filberts, 6 to 7 nuts</td>
</tr>
<tr>
<td>Buttery</td>
<td>unsalted Land 'O Lakes butter, approx. 20 g</td>
</tr>
<tr>
<td>Fruity</td>
<td>chopped overripe pineapple, approx. 20 g</td>
</tr>
<tr>
<td>Pungent Acidic</td>
<td>1.0% formic acid in distilled H₂O</td>
</tr>
<tr>
<td>Goaty</td>
<td>.1% butyric acid in distilled H₂O</td>
</tr>
<tr>
<td>Dirty</td>
<td>.1% isovaleric acid in distilled H₂O</td>
</tr>
</tbody>
</table>

1 All served in wine glasses covered with watch glasses
TABLE III-3. Aroma and flavor intensity standards for Cheddar cheese.

<table>
<thead>
<tr>
<th>Scale Value</th>
<th>Descriptor</th>
<th>Intensity Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aroma</strong>¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>slight</td>
<td>Saffola safflower oil (30 g)</td>
</tr>
<tr>
<td>7</td>
<td>moderate</td>
<td>Hi-C orange drink (18 g)</td>
</tr>
<tr>
<td>11</td>
<td>large</td>
<td>Welch’s grape juice (18 g)</td>
</tr>
<tr>
<td>15</td>
<td>extreme</td>
<td>Big Red gum (1 stick)</td>
</tr>
<tr>
<td><strong>Flavor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sour</td>
<td>.05% lactic acid in spring H₂O</td>
</tr>
<tr>
<td>6</td>
<td>Bitter</td>
<td>.05% caffeine in spring H₂O</td>
</tr>
<tr>
<td>6</td>
<td>Salty</td>
<td>.5% NaCl in spring H₂O</td>
</tr>
</tbody>
</table>

¹ Aroma standards were served in covered containers to trap volatile
TABLE III-4. Mean intensity ratings (0 = none, 15 = extreme) for aroma and flavor characteristics of three samples of commercial medium-sharp Cheddar.

<table>
<thead>
<tr>
<th>Intensity Rating Scores</th>
<th>Sample #</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Aroma Characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Overall Intensity</td>
<td>7.4</td>
</tr>
<tr>
<td>Buttery</td>
<td>5.4</td>
</tr>
<tr>
<td>Nutty</td>
<td>4.0</td>
</tr>
<tr>
<td>Fruity</td>
<td>2.8</td>
</tr>
<tr>
<td>Goaty</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>Flavor Characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Overall Intensity</td>
<td>6.9</td>
</tr>
<tr>
<td>Sour</td>
<td>4.9</td>
</tr>
<tr>
<td>Salty</td>
<td>4.9</td>
</tr>
<tr>
<td>Bitter</td>
<td>4.9</td>
</tr>
<tr>
<td>Sweet</td>
<td>1.9</td>
</tr>
<tr>
<td>Buttery</td>
<td>3.9</td>
</tr>
<tr>
<td>Nutty</td>
<td>2.3</td>
</tr>
<tr>
<td>Fruity</td>
<td>1.4</td>
</tr>
<tr>
<td>Goaty</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>T</td>
</tr>
<tr>
<td>------------------</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Aroma</strong></td>
<td></td>
</tr>
<tr>
<td>Overall Intensity</td>
<td>...</td>
</tr>
<tr>
<td>Buttery</td>
<td>...</td>
</tr>
<tr>
<td>Nutty</td>
<td>NS</td>
</tr>
<tr>
<td>Fruity</td>
<td>...</td>
</tr>
<tr>
<td>Pungent Acidic</td>
<td>...</td>
</tr>
<tr>
<td>Pungent Sulfur</td>
<td>...</td>
</tr>
<tr>
<td>Goaty</td>
<td>...</td>
</tr>
<tr>
<td>Dirty</td>
<td>...</td>
</tr>
<tr>
<td>Yeasty</td>
<td>...</td>
</tr>
<tr>
<td><strong>Flavor</strong></td>
<td></td>
</tr>
<tr>
<td>Overall Intensity</td>
<td>...</td>
</tr>
<tr>
<td>Sour</td>
<td>***</td>
</tr>
<tr>
<td>Salty</td>
<td>***</td>
</tr>
<tr>
<td>Bitter</td>
<td>...</td>
</tr>
<tr>
<td>Sweet</td>
<td>...</td>
</tr>
<tr>
<td>Buttery</td>
<td>...</td>
</tr>
<tr>
<td>Nutty</td>
<td>*</td>
</tr>
<tr>
<td>Fruity</td>
<td>...</td>
</tr>
<tr>
<td>Goaty</td>
<td>...</td>
</tr>
<tr>
<td>Dirty</td>
<td>...</td>
</tr>
<tr>
<td>Sulfur</td>
<td>...</td>
</tr>
</tbody>
</table>

1. T = temperature, A = age; P = panelist. * = P < 0.05; ** = P < 0.01; *** = P < 0.001; NS = non-significant; ... = not tested
### TABLE III-6. Interaction of temperature and age on overall aroma intensity

**Intensity means for all panelists**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Temperature (°C)</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.43)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.45)</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>7.39&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.34)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>8.22&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.66)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>6.71&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.66)</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>7.76&lt;sup&gt;de&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.61)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>9.38&lt;sup&gt;gh&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.33)</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>9.57&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.96)</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>10.92&lt;sup&gt;hi&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.40)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> a → i: means with the same letter are not significantly different

<sup>2</sup> Values in parenthesis are the standard deviations of reported means.
TABLE III-7. Mean sour and salty intensity scores for the effect of temperature and age on test Cheddar cheese

<table>
<thead>
<tr>
<th>TEMPORATURE (°C)</th>
<th>5</th>
<th>15</th>
<th>25</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sour</td>
<td>4.75a</td>
<td>5.89b</td>
<td>6.26b</td>
<td>6.89c</td>
</tr>
<tr>
<td>Salty</td>
<td>4.34a</td>
<td>4.85b</td>
<td>5.24c</td>
<td>5.38c</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AGE (days)</th>
<th>7</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sour</td>
<td>4.91a</td>
<td>6.21b</td>
<td>6.40b</td>
</tr>
<tr>
<td>Salty</td>
<td>4.50a</td>
<td>5.01ab</td>
<td>5.38b</td>
</tr>
</tbody>
</table>

1 a,b,c: within the same row, means with the same letter are not significantly different
IV. MODELLING OF TIME-TEMPERATURE EFFECTS ON BACTERIAL POPULATIONS DURING COOLING OF CHEDDAR CHEESE BLOCKS

ABSTRACT

Differences in cooling rate of Cheddar cheese from pressing (35°C) to aging temperature (3.5-12°C) has been reported to be responsible for flavor variation within a production lot. During aging, starter and non-starter bacteria contribute extensively to flavor quality. Temperature effects on these bacteria were quantified using cheese from a local processor. At day 1, starter counts were $8 \times 10^7$ CFU/g but as aging continued, starter counts decreased and non-starters (predominantly lactobacilli and pediococci) became dominant. At 35°C, starter counts reached $3 \times 10^6$ CFU/g by day 3 and were below $10^6$ CFU/g by day 5. At 25°C, 20°C, 15°C and 12°C, starter bacteria were below $10^6$ CFU/g by day 10, 20, 24 and 40, respectively. Non-starter counts, initially at $10^4$ CFU/g, reached $=10^8$ CFU/g at increasingly shorter times with higher temperatures. Kinetic analysis of non-starter growth in cheese and in a liquid medium suggested the possibility of diffusion growth limitations in cheese. Computer simulations for the growth of non-starter bacteria suggested that individual cooling of small blocks (18 kg) would reduce the contribution of non-starter counts to Cheddar cheese aging.
INTRODUCTION

The aging of traditional cheese types (Cheddar, Swiss, Gouda and Cheshire) has been defined as the controlled decomposition of a rennet coagulum of milk constituents (Lawrence and Gilles, 1987). As this process occurs, a balance of flavor and aroma compounds is formed which is strongly influenced by the microflora of the cheese. The microflora consists of the starter bacteria (*Lactococcus lactis* subsp. *cremoris*) which reach maximum levels during the cheesemaking process, and any non-starter bacteria present in the milk after heat treatment or introduced during the manufacturing process (Chapman and Sharpe, 1981). Both starter and non-starters have been found to contribute extensively to the flavor of the finished cheese (Law, 1984; Lawrence et al., 1984).

The starter microflora initially present in the curd dies out during aging (Law et al., 1976a,b, 1979); only the adventitious lactobacilli and pediococci increase in numbers during aging (Reiter et al., 1967; Law et al., 1976a,b; Sharpe, 1979). A delicate temperature balance is required to control the rate of growth of these bacteria during the initial phase of aging to achieve a desired cheese flavor (Fryer, 1982; Lawrence et al., 1983). Data by Law et al. (1979) and Fryer (1982) suggest that higher aging temperatures promote faster flavor development. However, elevated temperature conditions favor also the early die-off of starter bacteria prior to complete lactose utilization and open the way for formation of off-flavors by non-starter bacteria. The heterofermentative metabolism of lactose by non-starter bacteria produces such by-products as formic acid, ethanol, and acetic acid (Law, 1984).
Excessive levels of these compounds can impair the flavor balance of Cheddar cheese (Moskowitz, 1980).

Rapid cooling of cheese blocks to aging temperature is a primary means of control for microflora activity and promotes homofermentative metabolism (Fryer, 1982). Cromie et al. (1987) found that counts of lactobacilli increased more rapidly in cheese stored at higher temperatures (15 to 20°C) as compared to control cheese (8°C). This corresponds to the findings of Law et al. (1979) that non-starter bacteria increase more rapidly at 13°C than at 6°C. Quantitative comparisons have shown greater intensity for several sensory characteristics in test cheeses ripened at higher temperatures as compared to commercial samples from the same manufacturing plant (Chapter III). Many of these sensory characteristics could be considered defects because of their intensity.

An early survey of Cheddar cheese manufacturers showed that lack of control in the cooling step from pressing to curing was responsible for considerable variation in cheese flavor within lots (Vedamuthu et al., 1969). The temperature of the post-hoop cheese block ranges between a high of 35°C at pressing to the desired final aging temperature (3.5 to 12°C). For 290 kg (640 lb) block, a large temperature gradient is established within the block of cheese during the cooling period (Reinbold and Ernstrom, 1985). For 18.2 kg (40 lb) blocks, Conochie and Sutherland (1965) found a correlation between occurrence of cheese flavor defects and uneven cooling of blocks closely stacked on pallets. Miah et al. (1974) studied the effects of four 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
Some information is available on the effect of curing temperature on the microbiology of Cheddar cheese. However, little work can be found on microbial activity during the early aging stages when the cheese blocks are cooling. The data available are insufficient for modelling purposes. As shown in data reported by Reinbold and Emstrom (1985) and the industrial data shown in Figure IV-1 (Bailey, 1988), temperatures during the first eight days of cooling range from 5 to 35°C. The purpose of this study was to quantify the effect of this temperature range on starter and non-starter lactic acid bacteria. This information was examined though kinetic analysis and then used to simulate microbial growth patterns during cooling of 18.2 kg (40 lb) and 290 kg (640 lb) Cheddar cheese blocks. The effect of temperature on growth of non-starter bacteria was further analyzed using a liquid model system.

MATERIALS AND METHODS

Sample collection and storage

Cheddar cheese samples made with flash-heated milk (150°F, 15 s) were obtained from a major local manufacturer (Tillamook County Creamery Assn., Tillamook, OR). Six cheese batches were sampled directly after the pressing operation. Forty pound blocks were cut into 2.5cm x 5cm x 5cm (1" x 2" x 2") pieces of cheese. Each piece was vacuum shrink-wrapped in O₂-barrier film at the plant and immediately transported for placement in constant temperature incubators. The total time elapsed between cheese cutting and final temperature equilibration was less than
Cheese samples were randomly assigned to the various temperature treatment
groups.

Samples from batches #1 and 2, (January 1989) were stored at 5°, 15°, 25°, and
35°C. After preliminary analysis of these data, the 5°C samples confirmed the
expected microbial growth limiting conditions of storage at this low temperature. In
addition, this preliminary analysis showed that two additional temperatures, 12° and
20°C, were necessary for a more complete profile of microbial activity. Thus, samples
of batches #3 to 6 (June-July, 1989) were stored at 12°, 15°, 20°, 25°, and 35°C. The
experimental design consisted of two replications per batch and temperature treatment
for batches #1 and 2, and one replication per batch and temperature treatment for
batches #3-6. Sampling time was adjusted according to storage temperature.

**Proximate analysis**

A proximate analysis was completed at the beginning of the study for
production batches #3, 4, 5 and 6 (Bouzas et al., 1993). 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 (Official Method
16.259, AOAC, 1984). Duplicate fat determinations were conducted on 10 g ground
Duplicate protein determinations were made on 0.5 g ground cheese samples using the
macro-Kjeldahl method (Official Method 16.274, AOAC, 1984). Further chemical
analysis information on these batches can be found in Bouzas et al. (1993).
Microbiological assays

Eleven gram samples of cheese were diluted with 99 ml of sterile 0.1% peptone solution (Gilliland et al., 1984) and blended for 2 minutes in a Stomacher model 400 (Tekmar Co., Cincinnati, OH).

Starter culture. A combined plating and metabolic pattern characterization procedure was used to estimate starter counts. Appropriate dilutions of the cheese samples in 0.1% peptone solution were cultured in duplicate on M-17 agar (Terzaghi and Sandine, 1975; Gilliland et al., 1984; Sandine, 1989). Triplicate pour plates with an M-17 agar overlay were incubated for 48 h at 30°C. Ten colonies from plates with 25 to 250 colony forming units were selected at random for confirmation as starter bacteria. The substrate metabolic pattern of each selected colony was compared with those exhibited by the pure starter cultures (Marshall Products Div., Miles Inc., Madison, WI) forming the strain cocktail used for a particular production lot. For the determination of metabolic patterns, the ten selected colonies were streaked on plates of MRS agar (Difco Laboratories, Inc., Detroit, MI; DeMan et al., 1960; Gilliland et al., 1984). Plates were incubated anaerobically at 30°C for 48 h. Transfers from these plates were then cultured in MRS broth at 30°C for 24 h. A rapid CHL™ test kit (API Analytab Products Division, Sherwood Medical, Plainview, NY) was used for identification of metabolic patterns. The number of starter colonies \( (N_{\text{starter}}) \) was obtained by multiplying the counts observed on M-17 by the percentage of the ten isolates found to match the API-CHL™ metabolic pattern of one of the strains used in the starter culture cocktail.
Non-starter microflora. Non-starter lactic acid bacteria were defined as those microorganisms enumerated using LBS agar (Rogosa et al., 1951; Gilliland et al., 1984) to which 0.1% sodium azide was added (Sandine, 1989). Triplicate plates were incubated at 30°C for 8 d with an LBS overlay.

Liquid model studies. The growth of non-starter bacteria in the Cheddar cheese matrix was compared with growth in a liquid medium. Non-starter cultures were isolated from Cheddar cheese samples stored 8 d at 15°C to ascertain whether microbial activity in cheese is controlled by diffusion phenomena, i.e. the transport of nutrients to the cell and of metabolic waste to the cheese bulk. Ten gram samples of cheese were mixed with 20 ml of 0.1% peptone solution in a Stomacher. LBS broth (30 ml) was inoculated with 0.3 ml of this suspension and incubated at 32°C. The resulting culture was transferred to a modified MRS broth prepared with lactose instead of dextrose, and incubated at 32°C to a final density of $10^6$ to $10^7$ CFU/g. Triplicate side arm flasks (300 ml) containing 30 ml MRS broth, inoculated with 0.3 ml of the LBS culture, were incubated at 12°, 15°, 20°, 25°, and 35°C with shaking at approximately 300 rpm. Absorbance at 600 nm (Spectronic Model 20, Bausch & Lomb, Analytical Systems Div., Rochester, NY) was measured periodically until maximum cell density was reached.
Analysis of non-starter bacteria growth

Linear correlations. Specific growth rates for each cheese batch and temperature combination were calculated using linear correlations of non-starter counts during the exponential phase versus time.

Non-linear correlations. An alternative analysis of microbial kinetics can be based on the expression derived by Schoolfield et al. (1981) to estimate microbial growth and modified for the case of growth with negligible lag phase

\[
N = \frac{b}{1 + \left( \frac{b-N_0}{N_0} \right) e^{-0.056(1/T)} } 
\]

where \( N \) = cell concentration at time \( \theta \); \( N_0 \) = initial population; \( b \) = maximum cell population achievable; and, \( \mu_T \) = specific growth rate at temperature \( T \). Given that Eq.(1) is non-linear, a direct search procedure, the Complex method (Saguy, 1983), was used to find the parameters \( b \), \( N_0 \) and \( \mu_T \) (Almonacid et al., 1993, 1993a). A set of values for the parameters \( b \), \( N_0 \), and \( \mu_T \) was assumed and then Eq.(1) was used to search for the set minimizing the squared difference between observed and calculated non-starter counts.

Quantification of temperature effects. Specific growth rates obtained for each experimental temperature and the use of linear and non-linear correlations were analyzed using the Arrhenius model (Almonacid-Merino et al., 1993, 1993a,b). The temperature effect on specific growth rate (\( E_a \) values) was determined using a least
square procedure wherein \( \ln \mu_T \) served as the dependent variable and \( 1/T \) as the independent variable.

**RESULTS AND DISCUSSION**

**Proximate analysis**

It has been suggested that to produce Cheddar cheese of the most acceptable flavor, 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 desired range for premium cheese as suggested by Lawrence et al. (1984) are presented in Table IV-1. Values were not statistically different and fell within the premium cheese range except for FDM which was slightly above the recommended level (Bouzas et al., 1993). It should be noted that these differences correspond to processor operating conditions in response to consumer preferences and profitability.

**Starter bacterial counts**

Counts on M-17 agar commenced at the \((8.4 \pm 1.4) \times 10^7\) CFU/g range and essentially all colonies examined showed API-CHL™ metabolic patterns identical to those of the bacteria used in the starter culture cocktail. As aging continued, total M-17 counts remained in the \(10^7\) CFU/g range while colonies confirmed as starter
strains decreased and eventually disappeared at times depending on aging temperature (Table IV-2). For instance, at 35°C, starter counts dropped to 3x10^6 CFU/g by day 3 and were below 10^6 CFU/g by day 5. Decreasing temperature reduced the dying-off rate of the starter culture and also the growth rate of non-starter bacteria (Table IV-2).

**Non-starter bacteria counts**

Figures IV-2 and IV-3 show the growth of non-starter counts for Cheddar cheese ripened at temperatures in the 12 to 35°C range. At 15°C (Figure IV-2a), counts started at 10^4 CFU/g and increased to a maximum of 3x10^7 CFU/g at approximately 35 d of aging. For cheeses ripened at 20° to 35°C (Figures IV-2b,c,d), similar trends were observed, except that the maximum count was observed at successively earlier times. In cheese ripened at 12°C (Figure IV-3), it was difficult to distinguish between the exponential growth and the stationary phases. In cheese ripened at 5°C (data not shown), bacterial growth was markedly slower and maximum population was not observed after 120 d.

**Kinetic parameters for non-starter counts**

**Linear correlations.** Specific growth rates calculated on the basis of linear correlations for non-starter counts in the exponential phase are shown in Table IV-3 for each cheese batch and temperature combination. In spite of the expected variability between the non-starter populations for each batch the values obtained were remarkably similar considering that samples were obtained from a commercial plant.
The quantification of the temperature effect on the growth of non-starter lactic acid bacteria yielded an average $E_a = 16.2 \pm 3.6$ kcal/g-mol (Table IV-3). This value was lower than the typical range reported for this parameter, 20 to 60 kcal/g-mol (Taoukis, 1989), particularly if the slightly higher value for batch 6 is excluded yielding an average $E_a = 14.6 \pm 0.9$ kcal/g-mol (Table IV-3). This observation suggests that the growth of non-starter bacteria could be limited by kinetic processes other than growth but with lower $E_a$, e.g. diffusion of nutrients or metabolic waste products. Further evidence for diffusion-controlled growth was found in the analysis of the specific growth rates for non-starter bacteria in the liquid model system (Table IV-4). In this case, the specific growth rate ranged from 0.05 to 0.24 h$^{-1}$, while in the Cheddar cheese the values were on the order of 0.003 to 0.17 h$^{-1}$ (0.08 to 4.1 d$^{-1}$). The energy of activation for non-starter growth in the liquid media was 47 kcal/g-mol which is a typical value for microbial growth (Taoukis, 1989). The possibility for enzymic controlled growth, a kinetic process with $E_a = 10$ to 15 kcal/g-mol (Taoukis, 1989), possibly associated with lactose hydrolysis, was also eliminated by the high $E_a$ values observed during growth in the liquid model system (Table IV-4).

It should be noted that the microorganisms used in the liquid model analysis were obtained using an isolation procedure which might have favored the selection of non-starter bacteria with optimum growth at 32°C. Additional tests including starter cultures and non-starter isolation procedures at other temperatures and media will be included in future studies. This would be particularly important for the case of low
fat cheese because their higher moisture content would promote faster diffusion and more serious difficulties in controlling non-starter growth (Olson and Johnson, 1990).

**Non-linear correlations.** Table IV-5 summarizes the kinetic parameters obtained from the non-linear approach and were used to generate the predicted growth curves shown in Figure IV-2. The use of the growth model described by Eq.(1) has several advantages over the traditional linear regression analysis used for assessment of growth kinetics. First, no decision is required on the time period considered as exponential phase and all microbial counts from time 0 through the stationary phase can be used. Second, information in addition to specific growth rate ($\mu_T$) is obtained; namely, maximum cell counts ($b$) and initial cell counts ($N_0$). Finally, the availability of personal computers and calculation software negates the advantage of the mathematical simplicity of linear regression calculations.

It is interesting to note that the quantification of specific growth rate values using linear correlations for counts in the exponential phase is analogous to the graphic approach, i.e. determining the slope of the semilog plot of exponential phase counts. Both solutions attempt to minimize the difference between a theoretical line and the log-value of the microbial counts. On the other hand, the mathematical procedure based on Eq.(1) minimizes the difference between predicted and observed counts without a logarithmic transformation.

**Applications of non-starter kinetics.** The rapid cooling of cheese blocks toward the selected aging temperature serves as the primary means for control of microflora
activity and promotes homofermentative metabolism (Law et al., 1979; Fryer, 1982; Cromie et al., 1987). However, under industrial conditions, temperature control is not always easy to achieve (Reinbold and Ernstrom, 1985; Bailey, 1988). Temperature distributions observed during cooling of 290 kg (640 lb) (Reinbold and Ernstrom, 1985) and palletized 18.2 kg (40 lb) Cheddar cheese blocks (Figure IV-1) were used to estimate average non-starter counts during cooling of Cheddar cheese in commercial plants. The effect on non-starter bacteria populations of temperature differences between cheese locations within a 290 kg (640 lb) block, or between different 18.2 kg (40 lb) blocks palletized for easier handling and storage is shown in Figures IV-4 and IV-5, respectively. For 290 kg Cheddar cheese blocks, non-starter bacteria counts, initially at $2 \times 10^4$ CFU/g would reach in four days $3 \times 10^6$ CFU/g in the center and only $5 \times 10^4$ CFU/g in the outer layers of the block (Figure IV-3). Non-starter bacteria counts for the cooling of palletized 40 lb blocks would reach in the center $2 \times 10^7$ CFU/g in about 2 to 4 d depending upon block location on the pallet. On the other hand, counts on individually cooled blocks estimated as described by Almonacid et al. (1993, 1993b) would be much lower and reach by 4 d in the center only $2 \times 10^5$ CFU/g (Figure IV-5). This lower count highlights the advantages of the individual cooling of smaller size blocks as compared to 290 kg (640 lb) or palletized 18.2 kg (40 lb) blocks. This finding is consistent with recent studies on sensory and chemical changes as affected by temperature (Bouzas, 1991; Bouzas et al., 1991; Bouzas et al., 1993).
CONCLUSIONS

Temperature control affects the fate of starter and non-starter bacteria; a higher temperature results in a more rapid die-off of starter lactococci and favors a more rapid growth of non-starter bacteria. The early die-off of starter bacteria and the excessive growth of non-starter bacteria is known to lead to undesirable flavor characteristics. These findings support current research efforts and commercial processing changes towards individual cooling of smaller size Cheddar cheese blocks. Further studies are required to obtain more information on the block size effect and production site differences, particularly for the case of low fat Cheddar cheese. Finally, the apparent diffusion control of non-starter bacteria growth seems to further justify current industrial efforts to monitor tightly moisture content, and other Cheddar cheese composition parameters to ensure consistent flavor. It should be noted that microbial activities are not the only source of flavor differences and other factors are also important, e.g. the proteolytic activity of the coagulating enzyme which is also affected by the temperature profile in the cheese.

Finally, a parameter requiring further consideration are moisture gradients established during the Cheddar cheese cooling process (Reinbold et al., 1992a,b). Current efforts in our laboratories are directed at identifying the mechanisms and their relative importance to this moisture migration process.
Figure IV-1. Temperature distribution in a pallet holding 18.2kg (40lb) Cheddar cheese blocks during early aging (Bailey, 1988).
Figure IV-2. Non-starter bacterial counts on Cheddar cheese samples stored at 15-35°C. Symbols correspond to different batches. Lines are drawn using Eq.(1) with parameters listed in Table IV-5.
Figure IV-3. Non-starter bacterial counts for Cheddar cheese samples stored at 12°C. Symbols correspond to different experimental batches.
Figure IV-4. Estimated non-starter bacterial counts at center (A), intermediate (B) and surface (C) locations in 290 kg (640 lb) Cheddar cheese blocks under commercial cooling conditions (Reinbold and Ernstrom, 1985).
Figure IV-5. Estimated non-starter counts in the center of 18.2 kg palletized Cheddar cheese blocks cooled as shown in Figure IV-1 and for individually cooled blocks calculated as described by Almonacid et al. (1992a,c).
TABLE IV-1. Initial composition of Cheddar cheese samples

<table>
<thead>
<tr>
<th>Index</th>
<th>Range</th>
<th>Batch #</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/M</td>
<td>4.0 - 6.0</td>
<td>3 5.6</td>
</tr>
<tr>
<td>MNFS</td>
<td>52 - 56</td>
<td>4 6.0</td>
</tr>
<tr>
<td>FDM</td>
<td>52 - 55</td>
<td>5 5.3</td>
</tr>
<tr>
<td>pH</td>
<td>4.9 - 5.1</td>
<td>6 5.8</td>
</tr>
<tr>
<td>Protein %</td>
<td>40 - 40</td>
<td>---------</td>
</tr>
</tbody>
</table>

(1) S/M, salt-in-moisture; MNFS, moisture-in-non-fat-solids; FDM, fat-in-dry matter
(2) Values suggested by Lawrence and Gilles (1987). It should be noted that these values define a specific consumer preference.
(3) Mean of duplicate determinations
(4) Mean of triplicate determinations
TABLE IV-2. Starter (SB) and non-starter (NSB) bacterial counts (CFU/g) for Cheddar cheese samples stored at constant temperature

<table>
<thead>
<tr>
<th>T, °C</th>
<th>time, d</th>
<th>SB</th>
<th>NSB</th>
<th>time, d</th>
<th>SB</th>
<th>NSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>3</td>
<td>3x10^6</td>
<td>3x10^7</td>
<td>5</td>
<td>&lt;10^6</td>
<td>5x10^7</td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>8x10^6</td>
<td>1x10^7</td>
<td>10</td>
<td>&lt;10^6</td>
<td>2x10^7</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>1x10^7</td>
<td>6x10^6</td>
<td>20</td>
<td>&lt;10^6</td>
<td>2x10^7</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>3x10^7</td>
<td>8x10^6</td>
<td>24</td>
<td>&lt;10^6</td>
<td>1x10^7</td>
</tr>
<tr>
<td>12</td>
<td>24</td>
<td>2x10^7</td>
<td>10^4-10^5</td>
<td>40</td>
<td>&lt;10^6</td>
<td>10^4-10^5</td>
</tr>
</tbody>
</table>

(1) None of the 10 isolates matched the metabolic pattern of the bacteria present in the starter cocktail used for the particular batch.
TABLE IV-3. Kinetic parameters for the growth of non-starter lactic acid bacteria growth during Cheddar cheese aging.

Specific growth rate ($\mu_T$, d$^{-1}$)$^1$

<table>
<thead>
<tr>
<th>Batch #</th>
<th>12</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>35</th>
<th>ARRHENIUS MODEL $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$E_a$, kcal/g-mol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\ln \mu_o$</td>
</tr>
<tr>
<td>1</td>
<td>NT $^3$</td>
<td>0.43</td>
<td>NT</td>
<td>0.95</td>
<td>2.09</td>
<td>13.8</td>
</tr>
<tr>
<td>2</td>
<td>NT</td>
<td>0.59</td>
<td>NT</td>
<td>1.52</td>
<td>3.06</td>
<td>14.5</td>
</tr>
<tr>
<td>3</td>
<td>... $^4$</td>
<td>0.40</td>
<td>0.64</td>
<td>0.75</td>
<td>2.28</td>
<td>13.6</td>
</tr>
<tr>
<td>4</td>
<td>0.088</td>
<td>0.60</td>
<td>0.80</td>
<td>1.03</td>
<td>3.33</td>
<td>15.2</td>
</tr>
<tr>
<td>5</td>
<td>0.104</td>
<td>0.66</td>
<td>0.97</td>
<td>1.23</td>
<td>4.09</td>
<td>15.9</td>
</tr>
<tr>
<td>6</td>
<td>0.086</td>
<td>0.76</td>
<td>0.38</td>
<td>0.87</td>
<td>3.68</td>
<td>24.1</td>
</tr>
<tr>
<td>1 to 6</td>
<td>0.093±0.01</td>
<td>0.61±0.13</td>
<td>0.70±0.22</td>
<td>0.97±0.18</td>
<td>3.35±0.67</td>
<td>16.2±3.6</td>
</tr>
<tr>
<td>1 to 5</td>
<td>0.096±0.01</td>
<td>0.55±0.13</td>
<td>0.80±0.13</td>
<td>1.00±0.20</td>
<td>3.20±0.70</td>
<td>14.6±0.9</td>
</tr>
</tbody>
</table>

(1) Linear correlation, log transformation of non-starter counts in exponential phase (Statgraphics, STSC, Inc., Rockville, MD)
(2) Arrhenius model expressed as $\mu_T = \mu_o \exp[E_a/RT]$
(3) NT = not tested
(4) Poor correlation, data not included in analysis
TABLE IV-4.  Specific growth rate ($d^1$) for the growth of non-starter lactic acid bacteria in a liquid model system

<table>
<thead>
<tr>
<th>Temperature ($^\circ$C)</th>
<th>Specific growth rate ($h^1$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.055</td>
</tr>
<tr>
<td>15</td>
<td>0.084</td>
</tr>
<tr>
<td>25</td>
<td>0.115</td>
</tr>
<tr>
<td>35</td>
<td>0.241</td>
</tr>
<tr>
<td>Arrhenius model</td>
<td>$E_a$ value = 47 kcal/g-mol</td>
</tr>
</tbody>
</table>
TABLE IV-5. Kinetic parameters for the growth of non-starter lactic acid bacteria growth during Cheddar cheese aging based on an analysis of the entire growth curve.

<table>
<thead>
<tr>
<th>Batch #</th>
<th>b (fitted maximum counts)</th>
<th>N₀ (fitted initial counts)</th>
<th>μₜ (d⁻¹)</th>
<th>( Eₘ ) (kcal/g-mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 6</td>
<td>2.7 × 10⁷</td>
<td>38,100</td>
<td>0.283</td>
<td>21.7 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>2.1 × 10⁷</td>
<td>21,800</td>
<td>0.465</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.2 × 10⁷</td>
<td>66,700</td>
<td>0.691</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.8 × 10⁷</td>
<td>10,120</td>
<td>3.345</td>
<td></td>
</tr>
</tbody>
</table>

Parameters for Eq.(1) were determined using the Complex method (Saguy, 1983; Almonacid-Merino et al., 1993a,b).
CONCLUSION

This study quantified the relationship of time and temperature with the sensory and microbiological characteristics of commercially processed Cheddar cheese. In general, flavor and aroma characteristics intensified with increasing time and temperature. Exceptions were nutty and buttery aroma and flavor which tended to decrease in intensity as a function of time and temperature. Concurrently, microbiological growth rates increased as a function of time and temperature. Starter bacteria exhibited faster die-off with increasing temperatures. Non-starter bacteria reached maximum numbers more rapidly at higher temperatures.

Equations have been developed (Torres et al., 1992) for the sensory indicators studied here and also for the chemical indicators of aging quantified in a parallel study by Bouzas et al. (1993). All these equations have been factored into heat transfer models to predict the effect of block size, cooling conditions and aging times on Cheddar cheese characteristics (Bouzas et al., 1991; Torres et al., 1992). Further work would be beneficial to develop kinetic expressions for other cheese aging parameters such as flavor development in low-fat aged cheeses, and accelerated ripening strategies for aged cheeses.

From a microbiological standpoint, further research is required in the area of quantifying starter bacteria in the presence of competing microorganisms, particularly non-starter lactic acid bacteria. With no appropriate selected media available, characterization of starter bacteria was possible only to the extent that they were not over-populated by competing organisms on the initial pour plates. The option of using
cheese manufactured in a pilot plant was ruled out because populations of adventitious organisms would be dissimilar to commercial conditions. The option of using marker starters was disallowed in a commercial establishment. Therefore, growth characterization below $10^6$ was not feasible.
REFERENCES


Sandine, W.E. 1989. Personal communication. Department of Microbiology, Oregon State University, Corvallis, OR.


Yates, E. 1989. Personal communication. Tillamook County Creamery Assoc., Tillamook, OR.