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The role of fat in the flavor of cheddar cheese

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

DEDICATION

to

My Mother

INTRODUCTION

Although the flavor of all Cheddar cheese is not exactly the same, our senses of taste and smell distinguish Cheddar from other types of cheese. Unfortunately, typical Cheddar flavor cannot yet be detected or measured by physical or chemical methods. Also, it is not known how the characteristic Cheddar flavor is formed, although a great deal has been learned of the changes that take place in Cheddar cheese as it matures and of some of the agents responsible for these changes.

There are many difficulties in studying Cheddar cheese flavors. Major emphasis in most Cheddar cheese research has been on the effect of enzymes and bacteria on flavor development during ripening. Since ripening proceeds slowly with a multiplicity of interactions among ripening agents, it has not been possible to identify and study enough of these factors at one time to solve completely these interacting effects. The use of an artificial or simplified media to study the interactions often gives contradictory results, difficult to understand or to evaluate in terms of the actual conditions existing in ripening cheese.

Another complication is that it is difficult to study the effect of various possible ripening factors without evaluating the degree of flavor development. In addition to the usual difficulties with taste panels, few members agree on the designation of a cheese with the most typical Cheddar flavor.

The cheese has to be ripened for at least three or six months to develop the characteristic Cheddar flavor and this is time consuming.

To develop a good Cheddar flavor the cheese should be made in blocks of 20 pounds at least. This requires the labor of processing considerable volumes of milk or its constituents to introduce the required experimental variables.

But it is important to study Cheddar cheese flavor because knowledge of the components of Cheddar flavor are potentially of great economic interest to the cheese industry. By adding these components a more uniform product could be produced and the ripening period, "aging", which is usually 12 months, could be reduced. Also, it would be possible to give Cheddar flavor to imitation products, and the cost of Cheddar-like cheese to the consumer could be reduced by substituting vegetable oil for milk fat.

The object of this investigation was to study the precursors of Cheddar cheese flavor. This was done by modifying the fat used to make Cheddar cheese.

REVIEW OF LITERATURE

One of the best critical reviews on the flavor of Cheddar cheese was written by Mabbitt (57). Also, there were other recent reviews and articles on this subject by Marth (61), Day (16), Reiter et al. (77), Forss (24), and Fryer (27).

The compounds responsible for the typical flavor of Cheddar cheese have not been determined in spite of a considerable number of investigations in recent years. It appears that there are two schools of thought. One group tends to think of a single compound or a small group of compounds as being responsible for Cheddar cheese flavor, the same way that diacetyl is responsible for the flavor of butter. Some of the earliest workers in this field, Suzuki et al. (91), sought such a key flavor compound but identified only ethanol and ethyl acetate. The second school tends to believe that Cheddar cheese flavor is the result of a delicate balance between a large number of compounds which individually do not possess a flavor resembling that of Cheddar cheese. The latter idea seems to have predominated in recent years. Kristoffersen et al. (45), in agreement with Kosikowski and Mocquot (39), suggested that a balance of flavor components probably was more important than any one compound alone. Kristoffersen et al. (45) suggested that hydrogen sulfide and volatile fatty acids were among the components of a balanced flavor.

Harvey and Walker (30) found hydrogen sulfide in distillates from New Zealand Cheddar cheese but no other sulfur compounds. When the hydrogen sulfide was removed the characteristic Cheddar flavor decreased. Upon treatment with a reagent to remove carbonyls, the remainder of the characteristic Cheddar flavor was removed.

A mixture of fatty acids and carbonyls, including methional but not hydrogen sulfide, was prepared by Day et al. (17). Their mixture resembled Cheddar cheese but was not completely characteristic in aroma. Tasting procedures were not satisfactory because they could not find a suitable medium.

Another class of compounds represented by secondary butyl alcohol was found by Scarpellino and Kosikowski (80, 82). These compounds may be responsible in a way for the flavor of Cheddar cheese.

Thus, it appears that the theory that a balance of components is required to produce Cheddar flavor is closest to the mark. A determination of the correct balance, however, will require more exact quantitative and qualitative knowledge of the flavor components in Cheddar cheese.

Proteolysis in Cheddar Cheese

The pathway of proteolysis in Cheddar cheese has been somewhat clarified by the use of electrophoresis and chromatography to characterize the proteins and peptide fractions. There is agreement that the electrophoretic pattern of the cheese protein depends on the variety of the cheese and on its state of maturity (Annibaldi, 2; Lindquist and Storgards, 55).

Papers dealing with amino acids in cheese are less frequent now that the importance of these compounds in cheese flavor formation is better understood. Earlier suggestions that the specific flavor of Cheddar cheese might be caused by the presence of certain amino acids have not been confirmed. Early observations suggested that amino acids might make an important contribution to the flavor of Cheddar cheese because their

concentration in raw milk Cheddar cheese with full flavor greatly exceeded that in the milder pasteurized milk cheese (Kosikowski, 38). On the other hand, Baker and Nelson (6) showed, on the basis of organoleptic examinations made every four weeks during a 24-week curing period for two complete series of cheese, that histidine produced a definitely inferior cheese having a pronounced unnatural flavor due to the amino acid itself. Glycine, methionine, tyrosine, serine, glutamic acid, arginine, aspartic acid, and valine showed a possible tendency to increase the desirable flavor.

It was further shown that the amino acids present in the mature Cheddar cheese were, not unexpectedly, qualitatively similar to those found in casein (Block, 10). This was true for all the cheese types examined; however, some interesting differences were found. For example, in Cheddar cheese, orthinine occurs and is doubtless synthesized by the microflora (Mabbitt, 56). The pattern of free amino acids differs quantitatively from that of casein and reflects the fact that different amino acids are liberated at different rates and some may be further metabolized (Dacre, 14).

When amino acids were incorporated into fresh Cheddar cheese curd, in concentrations suggested by quantitative analysis, the mixture was reported by Dacre (14) to have no cheese flavor. This also was confirmed by tasting the peptide-amino acid fraction of Cheddar cheese isolated by ion exchange. These compounds had a broth-like flavor (Mabbitt, 56). With Cheddar cheese made from skim milk, broth-like flavor was predominant (Mabbitt and Zielinska, 58). A similar result was obtained by Silverman and Kosikowski (86) using a synthetic system. They found that certain

combinations of highly purified amino acids produced a cheese flavor, but it was characterized by a sweetness strongly resembling Swiss cheese flavor. Fatty acids alone, when incorporated in various combinations, produced unpleasant flavor of rancidity without any evidence of a cheesy flavor. When fatty acids and amino acids were incorporated together in concentrations close to those found by analysis of well ripened cheese, the flavor was pleasant, sharp, and had some, but not all, of the characteristics of typical Cheddar cheese flavor.

It is apparent, therefore, that amino acids contribute only a broth-like flavor to Cheddar cheese. This brothy flavor is not typical Cheddar flavor but does form an important background against which the typical flavor is produced. This supports an earlier hypothesis postulated by Mulder (65).

The further degradation of amino acids of the Cheddar cheese introduces some interesting possibilities. Amines and related compounds which may result from animo acid metabolism possibly play an important part in flavor development in Cheddar cheese (Silverman and Kosikowski, 85). Recent work by Kristoffersen and Gould (44) showed a close correlation between the concentration of hydrogen sulfide and Cheddar cheese flavor. They suggested that the gas is a product of the metabolism of lactobacilli.

Kristoffersen and Cole (42) studied Cheddar cheese quality as affected by the age of the milk and addition of certain compounds. They found that the addition of purine, hypoxanthine, xanthine, or uric acid to raw or pasteurized milk had a pronounced effect on the character of the cheese. With pasteurized milk cheese, increasing concentrations of these compounds resulted in cheese with curdy or corky body and with a flavor

progressing from sulfide-like towards fermented. With raw milk cheese, purine and hypoxanthine additions gave a short or mealy body and a sulfide-like flavor. Addition of uric acid resulted in cheese with a firm or corky body and a fermented flavor. These results indicate a possible relationship between inherent changes in milk, xanthine oxidase enzyme(s), and the quality of Cheddar cheese.

Recent work by Yamamoto et al. (100) showed that increasing the amount of rennet did not increase the free amino acids or promote flavor development in Cheddar cheese. When they raised the temperature of ripening from 10 C to 15 C, ripening of cheese was promoted and concentration of water-soluble nitrogen and free amino acids increased, but a fermented odor subsequently appeared and the cheese developed a strong taste with a poor texture. At 10 C cheese ripening was delayed, but the cheeses had no fermented odor and had a pleasant flavor which was superior to that of cheeses ripened at 15 C.

Walker (94) reported that hydrogen sulfide was the only sulfur compound which he could detect in cheese volatiles. Removal of this compound destroyed the original cheesy aroma.

Presumably hydrogen sulfide is formed by the breakdown of cysteine, cystein, and cysteic or methionine as suggested by Mabbitt (56). Although there is no good evidence that these amino acids decrease in concentration during ripening, the flavor threshold of sulfides, especially organic sulfides, is so low that a very small conversion of these amino acids could make a major contribution to Cheddar cheese flavor.

The production of hydrogen sulfide may be only an indicator of the simultaneous formation of other more potent and elusive sulfur compounds,

e.g., methyl sulfide. This compound merits special attention as stated by Patton et al. (73) and Jarczynski and Kiermeier (34).

Individual amino acids were studied by Keeney and Day (35) for the character of odor yielded by Strecker degradation. This reaction is the degradation of an amino acid to an aldehyde of one carbon atom less than the original amino acid. Methionine gave methional (3-methylthiopropanal) which has a cheesy-brothy flavor. In cheese, methional might be generated by a reaction such as this:

$CH_3SCH_2CH_2CHNH_2COOH$	+	$CH_3COCOOH$
methionine	↓	pyruvic acid
$CH_3SCH_2CH_2CHO$	+	$CH_3CHNH_2COOH + CO_2$
methional		alanine

In addition, the possible significance of methional in cheese flavor was demonstrated by Oro et al. (69). Its presence in cheese was confirmed also by Day and Keeney (18). Furthermore, 3-methylbutanal which has a malty aroma has been found in Cheddar cheese by Jackson and Morgan (33). The aldehyde could be derived from leucine by Strecker degradation. The Strecker degradation may be very important in Cheddar cheese flavor formation. A slow chemical interaction over a long period of ripening between amino acids and dicarboxyl compounds could lead to the production of flavorful aldehydes containing one carbon atom less than the original amino acids. These same aldehydes could be produced from amino acids by certain organsism, e.g., S. lactis through oxidative deamination (MacLeod et al., 59; MacLeod and Morgan, 60).

Lipolysis in Cheddar Cheese

Mabbitt and Zielinska (58) in 1956 reported that milk fat appears to be essential for the production of typical Cheddar flavor. When Cheddar cheese was made with skim milk, the resultant cheese after ripening had a broth-like flavor which resembled that obtained in the flavor tests with known mixtures of amino acids. In agreement, it was found in 1965 by Ohren and Tuckey (66) that Cheddar cheese made from skim milk acquired neither Cheddar cheese flavor nor typical body and texture. Moreover, the flavor of Cheddar cheese improved as the fat content of the milk increased. Concentration of free fatty acids was lowest in the skim milk Cheddar cheese, being approximately one-tenth the quantity in other cheese. The same authors (67) in 1969 added to the previous statement that only Cheddar cheese containing 50% fat or more on the basis of the dry matter developed a typical Cheddar cheese flavor, whereas Cheddar cheese with less than 50% fat did not.

Dacre (15) found that typical Cheddar cheese flavor was fat soluble, insoluble in water, and very volatile. The flavor was stable under neutral and acid condition, but was unstable under alkaline condition and could be decomposed by oxidizing or reducing agents. In agreement with Mabbitt and Zielinska (58), when cheese fractions were prepared by pressing and centrifuging Cheddar cheese, the typical Cheddar flavor resided mainly in the top fat phase, i.e., the flavoring substances were soluble in the fat layer. These flavoring compounds also have been found to dissolve in fat solvents. For instance, the typical Cheddar cheese flavor may be extracted from Cheddar cheese with diethyl ether, although the flavor has not so far been isolated from the extract.

Babel and Hammer (4) found that the Cheddar cheeses made with rennet paste used as the source of lipase were not lipolyzed. On the contrary, they were superior in flavor when compared to the control Cheddar cheese. The use of a small amount of rennet paste tended to mask the sour, acid flavor frequently encountered in the control Cheddar cheese. Addition of larger amounts of rennet paste resulted in a significant increase in the fat acidity of Cheddar cheese. All these cheeses showed lipolyzed flavor at some time during the ripening. The addition of mulberry juice to pasteurized milk did not improve the flavor of the resulting cheese, i.e., small amounts had little effect on the flavor while large amounts resulted in lipolyzed and unclean flavors. Irvine et al. (31) in 1954 improved the Cheddar flavor of pasteurized milk cheese. A strain of Geotrichum candidum isolated from raw milk curd, when inoculated into pasteurized milk 18 hr prior to cheese making, was found to promote a pattern of fatty acid liberation similar to that of raw milk cheese. In most cases the inoculated cheese received equal or higher flavor scores than the controls and appeared to give a rapid development of sharp Cheddar cheese flavor. However, inoculated milk previously pasteurized and homogenized yielded rancid flavor Cheddar cheese with very high volatile acid values and cheese fat high in acidity. The amount of C_6 and higher chain fatty acids in the inoculated pasteurized milk cheese was as high as in the raw milk Cheddar cheese. On the contrary, these acids were almost completely absent in the uninoculated pasteurized milk Cheddar cheese.

In later years, views on the importance of milk lipase in Cheddar cheese ripening have been reoriented. Albrecht and Jaynes (1) reported in 1955 that in addition to lipase active at alkaline pH, i.e., inactive in

Cheddar cheese, milk contains lipases which have optimum activity at around pH 5 and pH 6. Since the lipases or activators for the lipase enzymes are intimately associated with the milk casein (Dorner and Widmer, 23), there is a concentrating process during cheese making. Albrecht and Jaynes (1) demonstrated that a lipase could be quantitatively removed by precipitating the casein of skim milk with rennet or acid at its isoelectric point. It is likely that enzymes with different pH optima vary in their resistance to heat. It is clear, however, that much of the lipolytic activity is destroyed by pasteurization. Even the mild heat treatments which are used for cheese milk inactivate much of the lipolytic activity of milk (Irvine et al., 31; Sheuring and Tuckey, 84).

Recently it has become clear that bacteria which are present in relatively small numbers may be of great importance in Cheddar cheese ripening. It was shown that bacteria belonging to the genera Flavobacterium and Pseudomonas, which may grow in the milk during storage but which do not multiply in the Cheddar cheese, can produce lipolysis of the Cheddar cheese fat (Stadhouders and Mulder, 89). Furthermore, this lipolysis may still occur on a reduced scale even if the Cheddar cheese milk is pasteurized. Although the bacterial cells are destroyed by the heat treatment, their lipolytic enzymes are sufficiently heat stable to retain some activity (Stadhouders et al., 90). It also must be remembered that even nonlipolytic organisms, e.g., Lactobacilli, were shown to have lipolytic properties after autolysis (Peterson and Johnson, 74; Wolf, 99).

A correlation between the number of nongrowing lipolytic organisms in Cheddar cheese and the intensity of the flavor was found by Franklin and Sharpe (26). If lipolysis is important in Cheddar cheese flavor formation,

then so probably is the composition of the milk fat. The milk fat composition could be varied according to the composition of the feed from which milk is produced. A lowered plane of nutrition that causes the animal to use its body fat for milk fat production will result in a decrease in the volatile fatty acids content and an increase in the degree of unsaturation of the milk fat (Jack and Smith, 32). Other factors which might affect the milk fat composition are the stage of lactation and environmental temperature.

The significance of these fatty acids was studied by Kristoffersen and Gould (43). The rate of development of characteristic Cheddar flavor was greater in raw milk than in pasteurized milk Cheddar cheese. The characteristic flavor of high quality pasteurized milk Cheddar cheese was considered equal to that of raw milk cheese after about six months of ripening. The intensity of the characteristic flavor appeared to be related to the molar ratios of both C_5 and longer chain fatty acids and acetic acid to hydrogen sulfide. Cheddar cheese with ratios of C_5/H_2S x 100 from 1.5 to 5.0 and C_2/H_2S x 100 from 4 to 9 were rated highest in the characteristic of Cheddar flavor. Ratios outside these ranges were associated with lower characteristic flavor intensities. The concentrations of the hydrogen sulfide in the Cheddar cheese fluctuated during ripening, whereas those of free fatty acids generally showed a continuous increase.

Carbonyl and Volatile Compounds in Cheddar Cheese

Failure to attribute Cheddar flavor to any of the major constituents of ripe cheese and the conclusion of Dacre (15), that the components of

typical Cheddar flavor are very volatile and are present in trace amounts, led investigators to examine more closely the minor volatile components of cheese and, in particular, the carbonyl compounds. Dacre in 1955 (15) found that after the volatile fatty acids were removed the only substances identified in the flavor concentrate were ethanol, butyraldehyde, ethyl acetate, and ethyl butyrate. None of these compounds was related to Cheddar cheese flavor. In general it was found that the lower fatty acids, diacetyl and acetylmethyl carbonyl, contribute to Cheddar cheese flavor. In addition, Calbert and Price (12) reported in 1948 that a small amount of diacetyl seems to be essential in the typical flavor and aroma of Cheddar cheese.

Scarpellino and Kosikowski (81) tentatively indicated a relation between the flavor intensity and the quantitative and qualitative nature of the carbonyl compounds. Among the carbonyl compounds observed were three compounds which were unreported in Cheddar cheese chemistry literature. These carbonyl compounds were of chain lengths of four, six, and seven carbons. The four carbon compound has been identified as methyl ethyl ketone, and it existed at a relatively high concentration in all the aged Cheddar cheese.

Day and Keeney (18) identified some carbonyl compounds found in 16-month-old Cheddar cheese. These compounds were 3-methylthiopropanal (methional), formaldehyde, acetaldehyde, acetone, butanone, 2-pentanone, 3-methylbutanal, 2-heptanone, 2-nonanone, acetoin, and diacetyl. One of these compounds, 3-methylthiopropanal, appears to be the most important in Cheddar cheese flavor.

Bassett and Harper (7) in 1958 isolated and identified hydrazone derivatives of acidic and neutral carbonyl compounds. In the four individual cheeses studied, from 4 to 11 keto acids and from 2 to 8 neutral carbonyl compounds were present in the different cheeses. The keto acids identified were oxalacetic (trace), pyruvic, α-ketoglutaric, α-acetolactic, and α-ketoisovaleric. The neutral carbonyl compounds identified were acetaldehyde, acetone (trace), diacetyl (trace), and acetylmethyl carbinol (trace).

Patton et al. (73) in 1958 showed that the methyl ketones 2-heptanone, 2-butanone, and acetone are present in Cheddar cheese. They also reported formaldehyde, ethanol, acetoin, and diacetyl. One year later, Walker and Harvey (96) found a mixture of carbonyl compounds which they identified as acetaldehyde, acetone, acetoin, diacetyl, butane-2-one, pentan-2-one, heptan-2-one, nonan-2-one, undecan-2-one, and tridecan-2-one. Day et al. (17) in 1960 added formaldehyde, methylthiopropanal (methional), and 3-methylbutanal to the previous compounds but they did not detect acetoin or diacetyl.

The development of acetaldehyde and methyl ketones during the ripening of New Zealand Cheddar cheese was studied by Harvey and Walker (30). They found that one-day-old Cheddar cheese contained acetaldehyde and acetone together with traces of butanon-2-one and pentan-2-one. Heptan-2-one, nonan-2-one, and undecan-2-one appeared as the Cheddar cheese matured. The concentration of all these compounds progressively increased, especially after the typical Cheddar cheese flavor became apparent. They stated that these carbonyl compounds are present as such in Cheddar cheese and are not artifacts produced during steam distillation. This was in agreement with

Walker (94) and Walker and Harvey (96) who found the "cheesy" aroma of neutralized distillate from Cheddar cheese was destroyed when the carbonyl compounds were removed by the addition of 2,4-dinitrophenylhydrazine.

The importance of ketones to Cheddar cheese flavor development could be proven since Walker (95) in 1961 was able to produce a mild Cheddar flavor typical of 2-3-month old cheese by adding ketones, fatty acids, and thioacetamid (source of hydrogen sulfide) to fresh curd and curing at 12.8 C for three weeks. Similarly, Day et al. (17) described a mixture of carbonyls and fatty acids which included methional and 3-mercapto-propionic acid as having the most cheese-like aroma of the mixtures they tested. They suggested that their failure to produce a complete Cheddar aroma was due to the lack of accurate quantitative data as well as to incomplete qualitative data. In general, none of these attempts successfully duplicated Cheddar aroma by mixtures of known components of Cheddar volatiles. This might be because the correct combination of components has not been found yet, or to the lack of identification of one or more key aroma components.

Scarpellino and Kosikowski (80) developed a method of concentrating Cheddar cheese volatiles for gas chromatographic analysis. They detected 2-butanone, ethanol, diacetyl, and 2-butanol in Cheddar cheese volatiles.

A method using a Golay coated capillary column and a hydrogen flame ionization detector was developed by McGugan and Howsam (62). This system revealed about twice the number of components previously detected. Normal mature raw milk Cheddar cheese showed 40 to 50 volatile components. One cheese, with a slightly fruity flavor, showed 80 components. It was possible that a few of the peaks were due to rearrangement or decomposition

of cheese volatiles on the columns. Tentative identification based on this method has to be confirmed.

Marth (61) in his review in 1963 showed that the flavor components of Cheddar cheese flavor include carbonyl, nitrogenous, sulfur compounds, fatty acids, alcohols, salt, water, and unmodified cheese fractions.

The volatile components of the aroma of Cheddar cheese were analyzed by Wojtowicz and McGugan (98) in 1964. The analysis of the neutralized fraction on three gas-liquid chromatographic columns revealed peaks of 35 to 40 compounds of which 20 had a longer retention time than 2-octanone.

Kroger and Patton (50) developed a method which they believed preserved the original flavor components ratios more than most of the techniques applied so far in flavor research. They suggested that the ethanol level could be used as a criterion for detecting the maturity of Cheddar cheese. When its percentage in the vapor or head-space gas is found to be on a continuous decrease and reaches about 60 to 70%, then cheese should be consumed within 60 days. After that age, 270 days, most cheese tasted bitter and unclean.

The aroma fraction from the fat of high quality raw milk Cheddar cheese was isolated and identified by Day and Libbey (19). The aroma fraction was separated into approximately 130 components by the capillary column technique. Most of the major neutral components were characterized by the use of gas chromatography and mass spectrometry. These compounds included aldehydes; methylketones; primary, and secondary alcohol, and their esters; fatty acids; δ-lactones; and the isometric lactides of lactic acids.

Volatile components of Cheddar cheese were identified in 1966 by

Morris et al. (64). Other than hydrocarbons, 36 compounds were detected. Compounds found in two or more samples were acetone, butanone, 2-pentanone, 2-heptanone, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl acetate, ethyl butyrate, propyl acetate, dimethyl sulfide, 2-methyl-2-buten-1-al, and alkanoic acids of carbon numbers 2 to 6.

Bills et al. (9) showed that the concentrations of some components of Cheddar cheese varied widely among samples, whereas the concentrations of some other components remained relatively constant. They concluded it would be unreasonable to expect to find a good correlation between the flavor scores of Cheddar cheese and the concentrations of the relatively few volatile constituents they studied. Many less volatile compounds such as short chain fatty acids, and even nonvolatile compounds such as intermediates of protein degradation, are important to the total flavor. In fact, it is doubtful that the low concentrations of components such as acetone, which has a relatively high flavor threshold, contribute anything to the overall flavor of Cheddar cheese.

Three New Zealand Cheddar cheeses, each containing an appropriate quantity of a different synthetic triglyceride, triheptanion, or triundecanion, were made from milk of extremely low bacterial count (Lawrence, 51). These cheeses and two controls were ripened for nine months and developed a typical Cheddar flavor. Steam distillates of the samples of all the five cheeses contained extremely small quantities, ranging from 0.2 to 0.5 ppm, of C_6, C_8, and C_{10} methyl ketones while the contents of the odd-numbered C_3-C_{15} methyl ketones were 12-34 ppm. No significant amount of free fatty acid $<C_4$ was found. Their results

indicate that the flavorful C_5-C_{13} methyl ketones in New Zealand Cheddar cheese are derived by hydrolysis of esters of C_6-C_{14} β-keto acids and not by β-oxidation of the corresponding fatty acids.

McGugan et al. (63) studied the neutral volatiles in Cheddar cheese made aseptically with and without starter culture. Gas liquid chromatography and mass spectrometry detected the same volatiles in starterless cheese which had little or no Cheddar flavor as in cheese made with starter which had a characteristic Cheddar flavor. They found that methyl sulfide and dimethyl disulfide were the only compounds consistently detected in higher concentrations in the cheese made with starter than in the cheese made without starter. Also, Cheddar cheese made with starter developed a normal Cheddar flavor. The flavor of Cheddar cheese made in an open vat usually was stronger than that made in an aseptic vat (Reiter et al., 76).

McGugan et al. (63) used a total trapping technique on Cheddar cheese volatiles. They found that the combination of components recovered from the effluents of a chromatographic column did not have the Cheddar cheese-like aroma of the distillate vapors that were injected. This could be explained by the fact that one or more components essential in Cheddar cheese aroma might not have been eluted from the columns used and hence remained unidentified.

The volatile flavor materials recovered by vacuum distillation of natural Cheddar cheese, cheese slurries preheated to 49 C or 82 C during processing, and cheese powders produced by conventional and foam spray drying were studied by Bradley and Stine (11). Preheating the natural cheese prior to drying caused loss of some of the higher volatile compounds

and an increase in the number and relative concentration of other than volatile materials. This was expected since the higher the preheat treatment, the greater would be the loss of natural flavor compounds.

O'Keefe et al. (68) identified the following lactones in Cheddar cheese: delta C_{10}, C_{12}, C_{14}, C_{15}, and C_{16}; and gamma C_{12}, C_{14}, and C_{16}. Delta C_{18} was tentatively identified.

Liebich et al. (54) found that the relative concentrations of methyl ketones in Cheddar cheese are high, but not as predominant as in Roquefort cheese.

Free Volatile Acids in Cheddar Cheese

The volatile acids from acetic to capric contribute to the flavor of cheese. The lower members of this group, acetic and propionic, are products of bacterial fermentation, whereas the other members are mostly the result of lipolysis. The isolation and quantitative analysis of these acids have presented some problems. One of these difficulties is that acids up to and including caproic are water soluble, whereas caprylic and capric are not water soluble (Dixon and de Man, 21).

The flavor of the Cheddar cheese appeared to be related more to the ratio of free fatty acids and hydrogen sulfide concentrations (Kristoffersen and Gould, 44). Desirable Cheddar flavor occurred when these compounds were present in definite interdependent concentrations (40). Kristoffersen and Gould (43) found that the intensity of characteristic flavor of Cheddar cheese appeared to be related to the molar ratios of both C_5 and longer chain fatty acids and acetic acid to hydrogen sulfide. Also, they noticed that the concentration of hydrogen sulfide in Cheddar cheese fluctuated

during ripening, whereas those of free fatty acids generally showed continuous increase.

Patton (72) found that Cheddar cheese aroma cannot be completely simulated by an appropriate mixture of volatile fatty acids, but such a mixture apparently represents the backbone of the Cheddar aroma. Similar studies carried out by Liebich et al. (54) to simulate cheese flavor mixtures suggested that free fatty acids are the basis for any cheese flavor, but the characteristic aroma for a certain type of cheese appeared to be due to the proportions of the free fatty acids and other volatile components.

Acetic acid is the dominant volatile acid in Cheddar cheese (Kristoffersen and Gould, 44; Lawrence, 51; Scarpellino and Kosikowski, 82). This acid must be of a substantial importance to the unique aroma of Cheddar cheese (Patton, 72).

Lawrence (51) found that New Zealand cheese with typical Cheddar flavor contained either no detectable amount of fatty acids higher than C_4 or amounts that were not significant in terms of flavor threshold. This is in contrast with reports that fatty acids other than acetic and butyric play an important part in Cheddar cheese flavor (Forss and Patton, 25; Kristoffersen et al., 45). Lawrence (51) attributed this difference to New Zealand's Cheddar cheese being ripened at an appreciably lower temperature (45 F as opposed to about 55 F) and having a somewhat lower moisture content than Cheddar cheese made in Europe and North America.

The average concentration of the free fatty acids of Cheddar cheese expressed as mg of free fatty acid per kg of Cheddar cheese was determined

by Bills and Day (8) as follows: 2:0, 865; 4:0, 115; 6:0, 38; 8:0, 41; 10:0, 49; 12:0, 81; 14:0, 218; 16:0, 503; 18:0, 172, 18:1, 467; 18:2, 69; and 18:3, 40.

Ohren and Tuckey (66) observed that the concentration of free fatty acids was lowest in skim milk cheese. The free fatty acid values increased as the bacterial count of the raw milk increased, but the quality of the flavor was lowered. This increase of the free fatty acid appeared to be more closely related to bacterial count than to inactivation of lipase by pasteurization.

Dixon and de Man (21) estimated the free volatile acids in Cheddar cheese by gas liquid chromatography. The following results were given as a total volatile acid in mmoles per 100 g of cheese for typical samples of mild, medium, and old Cheddar cheese: 1.87, 2.16, and 2.31, respectively. The values for the acids of C_2, C_4, C_6, C_8, and C_{10} as mole percent of the total volatile acids were, respectively, for mild: 87.0, 7.7, 2.7, 1.2, 0.8; for medium: 82.6, 9.3, 2.6, 1.2, 1.5; for old: 65.5, 22.2, 6.0, 2.6, 2.6.

The results of the investigation of Ohren and Tuckey (67) showed that not only was it necessary to have an optimum quantity of free fatty acids plus acetates, but it also was important to have them in the proper ratio. In agreement, Patton (72) found acetate and volatile acid concentrations to be important factors contributing to Cheddar cheese. On the other hand, Kristoffersen (40) found no significant correlation between acetic acid and characteristic Cheddar flavor. After six months of aging, free fatty acids longer than C_5 showed a significant correlation with flavor.

Volatile Sulfur Compounds in Cheddar Cheese

Volatile sulfur compounds such as hydrogen sulfide, various mercaptans, and methional are produced in Cheddar cheese and may contribute significantly to the flavor of Cheddar cheese.

Kristoffersen and Nelson (49) examined the relationship of serine deamination and hydrogen sulfide production by Lactobacillus casei to Cheddar cheese flavor. Experimental Cheddar cheese was examined for sulfhydryl groups and free hydrogen sulfide. Both values of these components increased as the cheese matured. At six months the cheese containing the highest relative concentration of free H_2S received the highest flavor intensity score.

Keeney and Day (35) stated that methional from methionine was the most important flavor compound in Cheddar cheese. Methional imparts a Cheddar flavor in the presence of some other aldehydes derived from casein.

Kristoffersen and Gould (43) found that intensity of characteristic Cheddar flavor appeared to be related to the molar ratios of both C_5 and longer chain fatty acid and acetic to hydrogen sulfide. Cheese with ratios of $C_5/H_2S \times 100$ from 1.5 to 5.0 and $C_2/H_2S \times 100$ from 4 to 9 were rated highest in characteristic Cheddar flavor. Ratios outside this range were associated with lower characteristic Cheddar flavor intensities.

Dimethyl sulfide (CH_3-S-CH_3), observed in all good quality Cheddar cheese, was considered by Patton et al. (73) to be of obvious and direct importance to the aroma of Cheddar cheese.

Walker (94) and Walker and Harvey (96) studied the volatile sulfur compounds present in New Zealand Cheddar cheese, using gas entrainment and

steam distillation techniques. Hydrogen sulfide was the only sulfur compound which could be detected and, after removal of this compound from the gas stream or steam distillate, only a mixture of carbonyl compounds remained. They suggested that carbonyl and sulfur compounds are the two classes of compounds of major importance in Cheddar cheese flavor. However, they did not exclude other compounds, such as free fatty acids and amino acids, from playing a part in the general background flavor upon which the main cheese flavor is superimposed.

Kristoffersen and Gould (44) found a significant correlation at the 5 and 1% levels between characteristic Cheddar cheese flavor scores and pH, ammonia, and hydrogen sulfide after three months of curing. It was concluded that the flavor of Cheddar cheese appeared to be related more to the ratio of free fatty acids and hydrogen sulfide concentration than to any other compounds or combination of compounds they tested.

Kristoffersen et al. (46) showed that active sulfhydryl groups appeared in raw milk Cheddar cheese after one week of ripening, reaching a maximum value after one to three months of curing. Heating the milk used for making cheese delayed the appearance of active -SH groups in Cheddar cheese during curing. Generally they stated that the intensity of characteristic Cheddar flavor was related to the concentrations of active sulfhydryl groups of Cheddar cheese but not to bacterial count. Furthermore, the formation of active sulfhydryl groups in Cheddar cheese during curing may be the key to the development of characteristic Cheddar cheese flavor (Kristoffersen, 40). The formation of active sulfhydryl groups was impaired by heat treatment of the milk, relatively high redox potentials of the cheese, and presence of copper.

Kristoffersen and Harper (47) determined the volatile sulfur in Cheddar cheese by the use of S^{35}. Their results indicated that labeling of milk with S^{35} made it possible to determine the formation and the changes of the volatile sulfur compounds in Cheddar cheese during ripening. Their results establish definitely the formation of a variety of volatile sulfur compounds in Cheddar cheese and indicate that the production and concentration of these compounds are a function of heat treatment of the milk. Hydrogen sulfide and organic sulfide were present in all cheeses, and mercaptan and carbonyl compounds were present in all Cheddar cheese except the Cheddar cheese made from heated milk. The raw milk Cheddar cheese generally contained highest concentrations of hydrogen sulfide and carbonyl compounds, and the heated milk cheese contained lesser amounts of these materials and in inverse proportion to the intensity of heat treatment.

Libbey et al. (52) presented tentative evidence of the presence of methyl mercaptan in hard cheeses such as Cheddar. Further evidence for the presence of methyl mercaptan in high quality raw and pasteurized milk Cheddar cheeses was shown by Libbey and Day (53). This finding was confirmed by the work of Kristoffersen and Harper (47), mentioned previously.

McGugan et al. (63) showed that methyl disulfide and dimethyl sulfide were the only compounds consistently detected in higher concentration in the Cheddar cheese made with starter and having a characteristic Cheddar flavor, than in the Cheddar cheese made without starter and having little or no Cheddar flavor.

Acceleration of Cheddar Cheese Flavor Formation

A process to accelerate the ripening of Cheddar cheese was developed by Kristoffersen et al. (48). The Cheddar flavor can be obtained from fresh curd in 4 to 5 days. Basically, the process involved mixing two parts of 24-hour-old salted, unpressed Cheddar curd with one part of 5.2% sodium chloride solution and storage of the homogenous slurry at 30 C. They reported that the flavor of untreated slurry (no glutathione) became like that of mild Cheddar cheese. Addition of reduced glutathione, 10-100 ppm, resulted in fuller Cheddar cheese flavor. They concluded that the addition of reduced glutathione to the slurries resulted in increased formation of free C_4 and longer chain fatty acids, soluble protein, and bacterial growth.

Kristoffersen (41) studied the factors affecting flavor development in these semi-liquid Cheddar cheese preparations. He found that Cheddar-like flavor, compared with control slurries, resulted from the addition of 10 or 200 ppm reduced glutathione, 50 ppm cysteine hydrochloride, and at 35 C incubation temperature. The level of reduced glutathione affected only the rate of flavor development. Daily incorporation of air in the slurries was required for satisfactory flavor development. Lactic acid and active sulfhydryl groups increased in the slurries during storage.

Singh and Kristoffersen (87) later determined the effect of lactic cultures and the level of whey acidity at curd milling on the flavor quality of 7-day-old rapid cured Cheddar curd slurries with 100 ppm added reduced glutathione. They observed that the order of flavor preference was for slurries made from curd milled at 0.4%, 0.3%, and 0.2% developed acidity, respectively, regardless of lactic culture used. Besides that,

the lactic acid contents of the slurries increased during warm storage with the magnitude of increases inversely related to milling acidity of the curd.

It may be possible to reduce appreciably the time needed for cheese maturity by adding flavors produced by microorganisms. A Dutch patent on cheese aroma issued in 1966 to Unilever (92) indicated a useful approach to this concept. Microorganisms with the ability to split fat into products with the aroma of cheese were inoculated to a suitable medium under specific conditions. A Netherlands patent issued to Unilever (93) one year later claimed that synthetic Cheddar cheese flavor was more natural when 0.1 to 5 mg phenol, p-cresol, or guaiacol (0-methoxyphenol), and less than 100 mg of delta or epsilon lactones containing 4 to 22 C-atoms were added together with less than 600 mg of the necessary fatty acids. On the contrary, Badings et al. (5) found p-cresol to be responsible for a phenolic off flavor in Gouda cheese. This may support the flavor balance theory of Cheddar cheese mentioned previously.

MATERIALS AND METHODS

Manufacture of Cheese

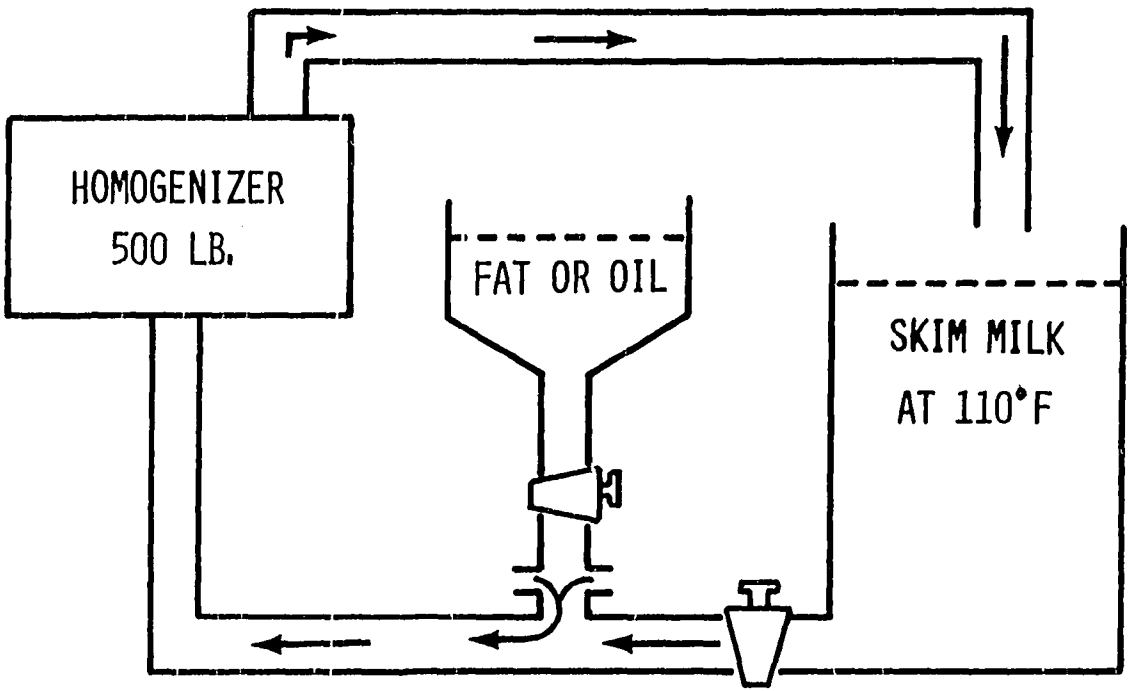
Eight lots of "Cheddar" cheese were manufactured in the Dairy Products Laboratory, Food Technology Department, Iowa State University. The cheese milk was produced by homogenizing various fats or oils into skim milk. To do this, the fat or the oil was fed along with the skim milk to a single stage homogenizer set at 500-lb pressure (see Figure 1). The skim milk was pasteurized at 62.8 C for 30 min. Just prior to homogenization the skim milk was heated to 43.5 C. After the homogenization process was completed, the milk was collected in 10-gal cans, cooled immediately in a brine tank, and stored for processing the next day. Each vat of cheese was made from 225 lb milk using the schedule and procedure outlined by Wilson and Reinbold (97). The same lot of skim milk was used for making each batch of Cheddar cheese.

Two lots of cheese were made using D-glucono-delta-lactone in place of starter according to the method of Dodson (22). Each vat was made with 20 lb of milk.

Preparation of cheese slurries

The modified method of Kristoffersen et al. (48) was used. Two parts of 24-hr-old salted unpressed Cheddar cheese were mixed with one part of 5.2% sodium chloride solution and the homogenous slurry was stored in sterilized jars at 30 C. These slurries were tasted daily, using one jar each time, up to 5 or 7 days. Reduced glutathione was added to the slurries at 100 ppm.

Figure 1. Schematic diagram for homogenization process


HOMOGENIZER
500 LB.
FAT OR OIL
SKIM MILK
AT 110°F

Preparation of natural milk fat

Natural milk fat was prepared by melting a sweet, unsalted butter in a 40 C water bath and adding an equal amount of distilled water. This mixture was stirred for about 5 min and then centrifuged to separate the milk fat. This process was repeated three times to get pure milk fat and remove milk solids.

Deodorization of natural milk fat

Deodorization of natural milk fat was done by steam distilling the milk fat under a pressure of 1 to 10μ at a temperature of 190 C for 5 hr. The distillate was collected in liquid nitrogen cooled traps. Appropriate amounts of distillate were homogenized with skim milk and deodorized milk fat and made into cheese to see how it would affect the Cheddar flavor.

Kaola and Kaomel

The Kaola and Kaomel were obtained from the Durkee Famous Food Company, Chicago, Illinois.

Esterification procedure for Kaola

The amount of tributyrin and tricaprion needed in Kaola to bring the levels of these fatty acids up to the levels found in milk fat was calculated. These amounts of tributyrin, tricaprion, and 0.05% sodium methoxide were added to the Kaola. The mixture was heated under vacuum at 60 C for 1-2 hr to randomize the fatty acids in the oil. Five percent aqueous acetic acid was added to stop the reaction. The oil was washed with Na_2CO_3 and then with distilled water until it was neutral. The oil was passed through a Rotafilm molecular still (A. F. Smith and Company) at room

temperature, a pressure of 5 to 10μ, and a rate of 20 drops/min. The oil was stored at 0 C until it was used.

Mineral oil

A bland mineral No. 13-3988 oil was supplied by Mineral Oil Refining Company, Dickinson, Texas.

Buttermilk solids

Buttermilk solids (State Brand Creamery, Mason City, Iowa) were homogenized with the cheese milk in a ratio of 1.16 g buttermilk solids per 100 g milk. This amount of buttermilk solids should contain about the amount of milk fat membrane material found in 100 g of milk.

Preparation of fat globule membrane

Most of the skim milk solids were removed from cream by washing it once with distilled water. After churning the buttermilk was collected and the butter granules were melted to separate the butter serum. Both the buttermilk and the butter serum were freeze dried. The freeze dried fat globule membrane material was added to the milk at the homogenization process. The amount added was calculated on the basis of the yield obtained and the amount of fat to be added to the skim milk for Cheddar cheese manufacture.

Gum acacia

The gum acacia U.S.P. Powder (J. T. Baker Chemical Company) was added to the milk at the homogenization process. The amount of gum acacia used was 1.5 g/100 g milk fat, because in their studies Chien and

Richardson (13) found 1.5 g of milk fat globule membrane material per 100 g milk fat.

Dimethyl sulfide

Dimethyl sulfide (K. and K. Laboratories, Inc.) was added to the oil in a concentration of 40 ppm.

Chemical Determination

Proteolysis determination

Proteolysis was determined by the modified Orange G method of Park et al. (70). About 0.1 g of cheese was weighed exactly and homogenized with 15 ml of Orange G dye solution with a test-tube homogenizer. After equilibration over night, the homogenates were centrifuged. The absorbance of the supernatant dye solution was measured as described by Hammond et al. (29) in a Beckman DU spectrophotometer in a special flow-through cuvette. The spectrophotometer was set to read zero at 475 nm when the cuvette was filled with 1:1 dilution of the dye solution.

Fat determination in milk and cheese

The Babcock method (Goss, 28) was used for fat determination in milk and cheese.

Moisture determination in milk and cheese

The Mojonnier method was used for moisture determination in milk. The oven was set at 100 C and the hot plate at 180 C. A 2 g sample was weighed directly into the 3-in diameter, 1-in high, covered dish which had just been passed through the oven and desiccator. The rest of the procedure was followed according to Goss (28).

Salt determination

The total chlorides were determined according to the A.O.A.C. method (3).

Judging the Cheeses

The cheeses were judged for Cheddar flavor at the ages of 3, 4, 5, 6, 9, and 12 months by 20 judges, and at least 13 judges were present each time. All taste panels were held at the same time of day, with each judge having his own booth. Lighting conditions and the temperature of the samples and the tasting room were closely controlled. Only Cheddar flavor was considered in the judging, using a score sheet as shown in Figure 24 in the Appendix.

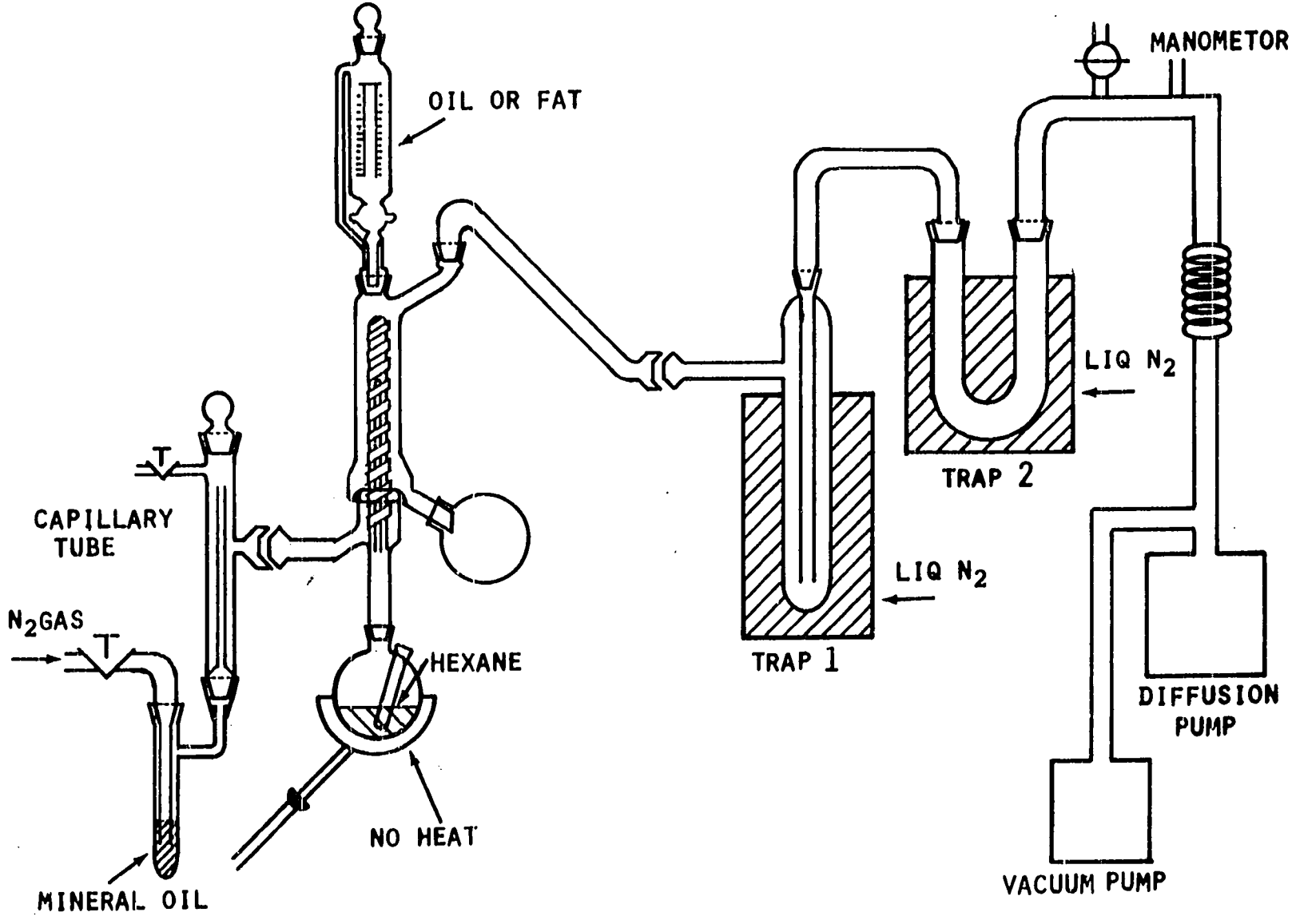
Organoleptic Analysis of Cheese Volatiles

Identification of Cheddar cheese flavor

The method of Libbey et al. (52) was used to extract cheese fat. About 200 g of cheese were ground and packed into 50-ml stainless steel centrifuge tubes. The tubes were centrifuged in a Sorrall centrifuge Model RC2-B at 30,000 gravity for 20 min at 40 C. The cheese fat was distilled in a Kontes micromolecular still at a very slow flow rate (10 drops/min). The temperature was maintained at 40 C by refluxing commercial hexane in the heating finger of the still. A stream of nitrogen gas was used to sweep the volatiles to be collected into a glass trap cooled with liquid nitrogen. The vacuum was maintained at 1 to 10μ. A schematic diagram for this apparatus is presented in Figure 2.

It is fairly generally agreed that an important and characteristic portion of Cheddar flavor is found in the volatiles dissolved in the fat.

Figure 2. Molecular still


OIL OR FAT
MANOMETOR
LIQ N_2
TRAP 2
LIQ N_2
TRAP 1
CAPILLARY TUBE
N_2GAS
HEXANE
NO HEAT
MINERAL OIL
DIFFUSION PUMP
VACUUM PUMP

The above method isolates and concentrates the volatiles under mild conditions, which minimizes opportunities for alteration or loss of components.

The volatiles were transferred to a stainless steel U trap by means of the apparatus shown in Figure 3. The cartridge heater was used to warm the inlet of the stainless steel trap to prevent plugging by freezing volatiles. The glass trap was warmed and a stream of nitrogen gas was used to transfer the volatiles to the stainless steel trap. The vacuum was maintained at about 0.050 mm. About 1 hr was required for this transfer.

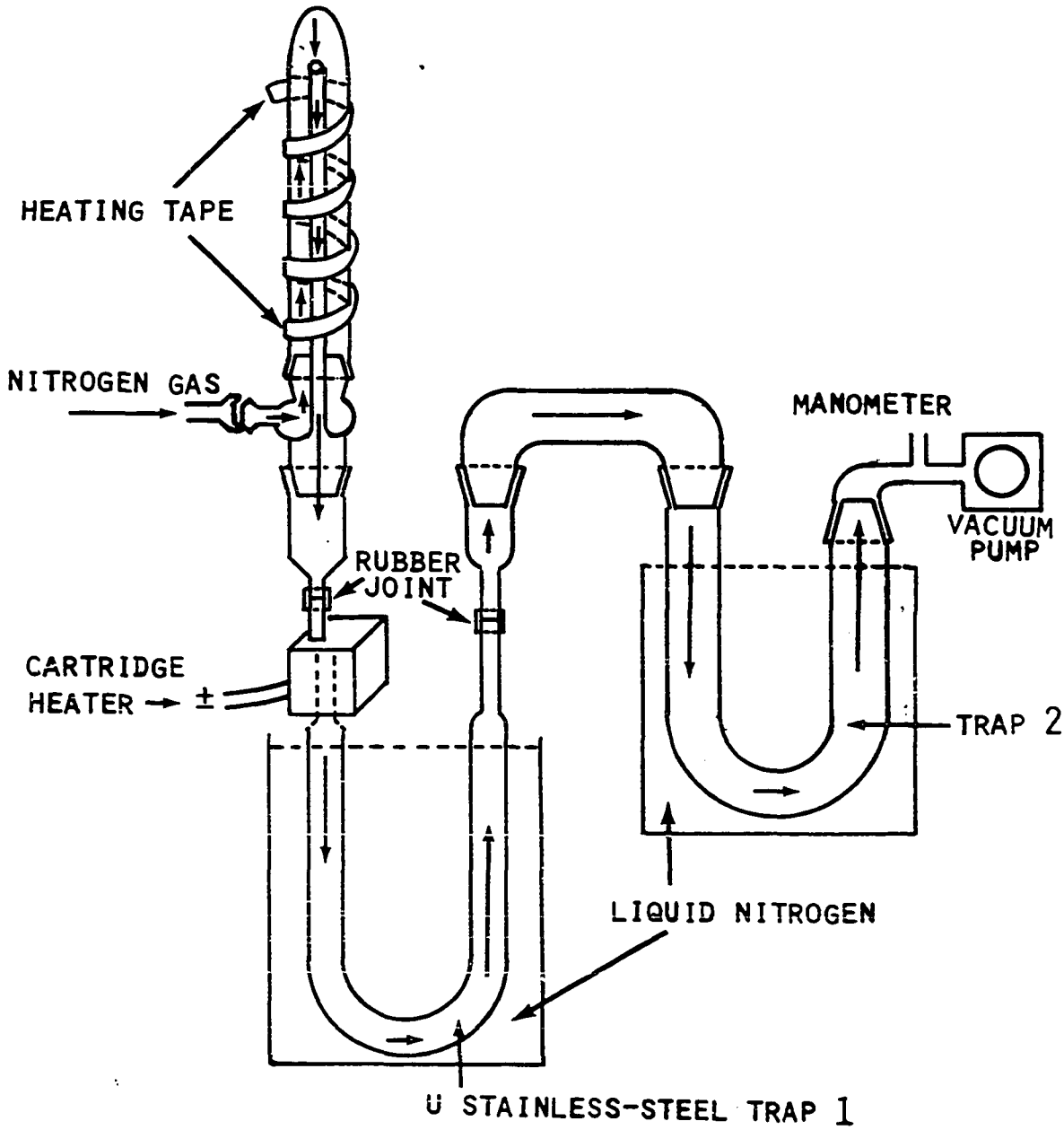
The stainless steel trap was connected to the injection ports of a chromatograph, Beckmann Model GC-5. Immediately after the injection, the stainless steel U tube was heated by alcohol flame for 5 min. The gas chromatographic conditions were: helium flow rate 20 ml/min, rise time 16 min, temperature programmed from 80 C to 160 or 170 C, prerise time 5 min, detector 260 C, and injection port 210 C. Columns used were: 30% butanediol succinate on 100/120 mesh Chromosorb P and 30% apeizon L on 100/120 mesh Chromosorb W, AW-DMCS. Standards consisting of a mixture of normal fatty acids of C_{2-6} and methyl ketones of $C_{3-9,11}$ were injected each time after the sample injection. A splitter with a ratio of 1:1 was attached to the end of the column. Half of the gas was conducted to a flame detector and half was vented for organoleptic examination.

Four to five panelists organoleptically evaluated the gas chromatographic eluant, and their responses were recorded. At least two panel members had to report an aroma before it was recorded as being present.

Preparation of precolumn reactors

Reaction columns to remove compounds with certain functional [illegible]s were prepared by a modification of the method of Regnier and Huang (75).

Figure 3. Volatiles transfer apparatus


HEATING TAPE
NITROGEN GAS
RUBBER JOINT
CARTRIDGE HEATER
MANOMETER
VACUUM PUMP
TRAP 2
LIQUID NITROGEN
U STAINLESS-STEEL TRAP 1

A small piece of 1/8-in stainless steel column was packed with the reagents and was connected to the injection port at one end and to the regular column at the other end. The reagents were prepared as described by Regnier and Huang (75). An 0.5% (w/w) boric acid coated column support was prepared by dissolving 10 mg of boric acid in 2 ml of water on a steam bath. After the boric acid dissolved, 1 ml of acetone was added to the solution and the resulting mixture added to 2 g of 30% butanediol succinate on 100/120 mesh Chromosorb P in a 500-ml round bottom flask. The slurry was mixed thoroughly and the solvents removed by rotatory vaporizer under vacuum. One hundred mg of the 0.5% boric acid coated support was used in the precolumn reactor. Sodium borohydride, 98% ($NaBH_4$), J. T. Baker Chemical Company, precolumn reactors were prepared with 200 mg pure reagent.

Statistical Analysis

Panel selection

The cheese types were ranked according to their flavor scores: 1 or 2 or 3 and 1 or 2 or 3 or 4 depending on the number of the types in each experiment. The type with the highest flavor score was given the highest ranking order. Then, 3x3 or 4x4 joint frequency distribution chi-square for type vs rank was calculated for each experiment. The chi-square values were divided by 4 or 9 degrees of freedom depending on the number of types in each experiment. An analysis of variance (AOV) table was presented; experiment, judge, and error were in the model. The chi-square measures were adjusted for experiment and the t-test was calculated

to compare judges. A multiple linear regression program by Kennedy (36) was used to compute the analysis of variance table and t-values.

Flavor score analysis

The flavor scores from experiment 1 through 6 were pooled together and analyzed by an analysis of variance and multiple comparison of adjusted means according to Snedecor and Cochran (88). The model used was

$$Y_{ijkl} = u + (\text{experiment})_i + (\text{length of ripening period})_j + (\text{type})_k + (\text{judge})_l + (\text{error})_{ijkl}; \; i=6, \; j=6, \; k=10, \; l=16.$$

An AOV table and a multiple comparison of adjusted means for experiments, length of ripening period, types, and judges were calculated.

The interaction between experiment and length of ripening period, and length of ripening period and type, were tested according to Snedecor and Cochran (88).

The model used to test the interaction between experiment and length of ripening period was

$$Y_{ijkl} = u + (\text{experiment})_i + (\text{length of ripening period})_j + (\text{type})_k + (\text{judge})_l + (\text{experiment x length of ripening period})_{ij} + (\text{error})_{ijkl}; \; i=6, \; j=6, \; k=10, \; l=16.$$

The model used to calculate the interaction between length of ripening period and type was

$$Y_{ijk} = u + (\text{experiment})_i + (\text{length of ripening period})_j + (\text{type})_k + (\text{length of ripening period x type})_{jk} + (\text{error})_{ijk}; \; i=6, \; j=6, \; k=6.$$

An analysis of variance table was calculated to test the difference between milk fat cheese which was used as a control for our study against conventional cheese with the same age. The analysis of variance table calculation was conducted according to Snedecor and Cochran (88). The model used was

$$Y = u + (\text{type})_k(\text{experiment})_i(\text{type})_k;\ i=3,\ k=2.$$

Experiments 7 and 8 were analyzed separately for analysis of variance. The model used for experiment 7 was

$$Y_{jkl} = u + (\text{length of ripening period})_j(\text{type})_k + (\text{judge})_l + (\text{length of ripening period x type})_{jk} + (\text{error})_{jkl};$$
$$j=5,\ k=4,\ l=13.$$

The model used for experiment 8 was

$$Y_{jkl} = u + (\text{length of ripening period})_j + (\text{type})_k(\text{judge})_l + (\text{length of ripening period x type})_{jk} + (\text{error})_{jkl};$$
$$j=3,\ k=2,\ l=13.$$

RESULTS AND DISCUSSION

Preliminary Studies with Cheese Slurries

At first we tried to use the slurry method of Kristoffersen et al. (48) for rapid development of Cheddar cheese flavor to test the role of fat in flavor formation. To test the effect of starter and glutathione on the flavor two lots of cheese were made using D-glucono-δ-lactone in place of starter, according to the method of Dodson (22). Each vat was made with 20 lb milk prepared by homogenizing milk fat into skim milk. When the curd was 24-hrs old, it was prepared according to Kristoffersen et al. (48) except that the homogenous slurry was stored in sterilized jars at 30 C. When the effects of the addition of reduced glutathione were tested it was added in a concentration of 100 ppm. These slurries were tasted daily, using one jar each time, for 5 or 7 days. Table 1 shows the average of the flavor score of ten judges.

Table 1. Flavor score[a] of cheese slurries

Cheese sample no.	Culture	Glutathione	Cheese slurries aged in days 1	2	3	4	5
1	+	+	1.75	1.12	1.00	1.37	0.33
2	+	-	0.50	0.57	0.62	0.87	0.50
3	-	+	1.37	0.85	0.25	1.00	0.50
4	-	-	0.37	0.28	0.25	0.50	0.50

[a]Cheddar flavor absent, 0; slight, 1; definite, 2; pronounced, 3; very pronounced, 4.

The data in Table 1 show that the reduced glutathione raised the average of the flavor scores both with or without using starter culture. Kristoffersen et al. (48) claimed that the flavor of slurry with no glutathione became like that of mild Cheddar cheese. Our results do not support this claim, but the Cheddar flavor may have been masked by the growth of undesirable organisms.

A second lot of Cheddar cheese was made using D-glucono-δ-lactone according to the method of Dodson (22). A starter culture was added to all samples. The fat or oil used in this lot was butter fat, deodorized butter fat, and mineral oil. Each vat was made from 20 lb milk. The 24-hr-old salted unpressed curd was treated according to Kristoffersen et al. (48) and the flavor scores are presented in Table 2.

Table 2. Flavor score[a] of cheese slurries

Type of fat or oil	Glutathione	Cheese slurries aged in days						
		1	2	3	4	5	6	7
Milk fat	-	0.50	0.71	1.13	0.67	0.71	1.38	1.11
Milk fat	+	1.62	1.29	2.00	1.17	1.71	1.75	1.89
Deodorized milk fat	-	0.75	0.43	0.50	0.67	0.86	1.00	1.44
Deodorized milk fat	+	1.25	1.00	1.50	1.83	1.42	1.88	1.67
Mineral oil	-	0.50	0.57	0.88	1.00	0.71	0.75	1.78
Mineral oil	+	0.75	0.86	0.75	1.00	1.00	1.13	1.22

[a]Cheddar flavor absent, 0; slight, 1; definite, 2; pronounced, 3; very pronounced, 4.

Table 2 shows again that the reduced glutathione raised the average flavor scores in most cases in agreement with Kristoffersen et al. (48).

The addition of the reduced glutathione to mineral oil cheese had less effect on the flavor and took longer to make its effect noticeable. In general, milk fat with glutathione was similar to deodorized milk fat with glutathione in flavor development. Mineral oil cheese tended to score lower than cheese made with milk fat or deodorized milk fat, especially in the first three days of storage.

The panelists scored all the samples low in Cheddar flavor and there was considerable disagreement among the judges. Some consistently saw no resemblance between the flavor of the slurries and Cheddar cheese. These differences may have been caused by individual difference in the perception and concept of what constitutes Cheddar flavor. The high salt and moisture content of the slurries may have been distracting influences.

We judged slurry method for accelerating Cheddar flavor as unreliable for our purposes. We decided to obtain valid results we had to use the procedure for making Cheddar cheese and allow it to ripen naturally.

Studies with Ripened Cheese

The oils and fats used for making the cheese were as follows:

Experiment 1

1. Milk fat
2. Kaola
3. Kaomel

Experiment 2

1. Milk fat
2. Kaola
3. Kaomel

Experiment 3

1. Milk fat
2. Milk fat plus buttermilk solids
3. Deodorized milk fat
4. Deodorized milk fat containing an appropriate amount of the distillate

Experiment 4

1. Milk fat
2. Kaola
3. Esterified Kaola

Experiment 5

1. Milk fat
2. Mineral oil
3. Mineral oil containing 40 ppm dimethyl sulfide

Experiment 6

1. Milk fat
2. Deodorized milk fat
3. Deodorized milk fat containing 40 ppm dimethyl sulfide
4. Deodorized milk fat containing an appropriate amount of the distillate

Experiment 7

1. Milk fat plus fat globule membrane
2. Milk fat plus heated fat globule membrane
3. Mineral oil plus fat globule membrane
4. Mineral oil plus heated fat globule membrane

Experiment 8

1. Milk fat
2. Milk fat plus gum acacia

The cheeses in experiment 1 to 6 were ripened 12 months, but cheeses in experiments 7 and 8 were ripened only 9 and 6 months, respectively. Table 20 in the Appendix gives the determination of fat, moisture, and total solids for the milk used for manufacturing cheese, and also the determination of fat, moisture, solids, fat on dry basis, and salt for Cheddar cheese immediately after the pressing period.

Panel selection

The ability of the 20 judges to order the flavor scores consistently by rank as the samples ripened was tested by using adjusted chi-squares. It was assumed the ranking order of the cheese types should not change during the ripening period and the experimental results generally confirmed this. Table 3 shows the analysis of variance of adjusted chi-squares for the judges.

Table 3. Analysis of variance of chi-square for the judges

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	116	376.36		
Regression	25	265.33	10.61	
Judge	19	209.71	34.95	28.65**
Experiment	6	50.70	2.67	2.19
Error	91	111.03	1.22	

**Significant at the 1% level.

It is clear from Table 3 that the experimental effect was not significant at the 5% level with an F-ratio value of only 2.19, but the judge effect was significant at the 1% level with an F-ratio value of 28.65. Because of this a t-test was made to compare the judges.

Table 4 shows the comparison of judges after adjusting for experiments.

Table 4. Comparison of judges after adjusting for experiments

Judge	Coefficient	T-value	Standard error	Standard coefficient
1	-0.033	-0.079	0.410	-0.005
2	-0.729	-1.646	0.443	-0.112
3	-0.933	-2.273*	0.410	-0.151
4	0.260	0.535	0.486	0.038
5	-0.054	-0.132	0.410	-0.009
6	0.293	0.714	0.410	0.047
7	-0.153	-0.372	0.410	-0.025
8	1.847	4.502**	0.410	0.299
9	0.940	2.122*	0.443	0.145
10	0.130	0.317	0.410	0.021
11	-0.169	-0.382	0.443	-0.026
12	0.046	0.086	0.543	0.006
13	-0.353	-0.859	0.410	-0.057
14	-0.077	-0.187	0.410	-0.012
15	-0.672	-1.236	0.544	-0.092
16	0.149	0.363	0.410	0.024
17	0.315	0.503	0.628	0.040
18	-0.895	-2.182*	0.410	-0.145
19	0.643	1.026	0.627	0.081
20[a]	--	--	--	--

[a]Judge 20 used as a contrast.

**Significant at the 1% level.

*Significant at the 5% level.

The judges were compared against judge 20, who was selected randomly. T-values in this table with a positive result indicate a better distribution and with a negative result indicate a poorer distribution relative to judge 20. Judges 3 and 18 were significantly lower than judge 20 at the 5% level, but judges 2 and 15 were not significantly lower. It was decided arbitrarily to remove the data of the four least consistent judges. These four are judges 2, 3, 15, and 18 with a t-value of -1.646, -2.273, -1.236, and -2.182, respectively.

Flavor scores in experiments 1 to 6

Table 5 shows the analysis of variance of flavor scores for experiments 1 to 6. The model used for this analysis was

$$Y = u + (\text{experiment})_i + (\text{length of ripening period})_j + (\text{type})_k + (\text{judge})_l + (\text{error})_{ijkl}; \; i=6, \; j-6, \; k-10, \; l=16.$$

Table 5. Analysis of variance of flavor scores for experiments 1 to 6

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	1221	1152705.46		
Experiment	5	58460.37	11692.07	19.09**
Length of ripening period	5	30480.26	6096.05	9.95**
Type	9	313855.61	34872.85	56.93**
Judge	15	57866.68	3857.78	6.30**
Error	1187	727058.38	612.52	

**Significant at the 1% level.

Table 5 shows that the effect of variables experiment, length of ripening period, type, and judge are all significant at the 1% level with F-ratios of 19.09, 9.95, 56.93, and 6.30, respectively. The F-ratios

indicate that the differences due to experiment, length of ripening period, and judge are significant at the 1% level, but their values are relatively small when compared to the effect of type which had an F-ratio of 56.93.

The effects of the ripening period were tested by a multiple comparison of adjusted means of flavor scores. Table 6 shows a multiple comparison of 3 months against 4, 5, 6, 9, and 12 months ripening period.

Table 6. Multiple comparison of adjusted means of flavor scores for the length of ripening periods for experiments 1 to 6

Comparison of ripening period	Difference in adjusted mean x 10	T-value	Standard error x 10	Standard partial regression coefficient
3 mo vs 4 mo	1.99	0.83	2.40	0.02
3 mo vs 5 mo	4.32	1.86	2.32	0.05
3 mo vs 6 mo	9.94	4.12**	2.41	0.12
3 mo vs 9 mo	9.61	3.80**	2.53	0.11
3 mo vs 12 mo	14.64	5.98**	2.45	0.17

**Significant at the 1% level.

There was no significant difference between 3 months and 4 months or 5 months. The remainder of this comparison was significant at the 1% level. This indicated that the cheese had developed more flavor as it matured.

The effect of judges was tested by testing judge 1 against the other selected judges. Table 7 shows that the differences of judge 1 against judges 6 and 13 were significant at the 1% level. The difference between judge 1 and judge 9 was significant at the 5% level. This indicates that only 3 judges out of 15 were significantly different as compared to judge

1. This also indicates that these selected judges were consistent in scoring the cheese.

Table 7. Multiple comparison of adjusted means of flavor scores for judges for experiments 1 to 6

Comparison of judges	Difference in adjusted mean x 10	T-value	Standard error x 10	Standard partial regression coefficient
Judge 1 vs 4	-4.85	-1.22	3.98	-0.04
Judge 1 vs 5	3.45	1.01	3.40	0.03
Judge 1 vs 6	-13.32	-3.92**	3.40	-0.13
Judge 1 vs 7	-5.32	-1.40	3.79	-0.04
Judge 1 vs 8	-5.19	-1.46	3.54	-0.05
Judge 1 vs 9	7.78	2.32*	3.35	0.08
Judge 1 vs 10	-4.21	-1.12	3.77	-0.03
Judge 1 vs 11	-1.73	-0.44	3.93	-0.01
Judge 1 vs 12	-9.76	-2.02	4.82	-0.06
Judge 1 vs 13	-16.42	-4.61**	3.56	-0.14
Judge 1 vs 14	3.95	1.10	3.60	0.03
Judge 1 vs 16	-2.60	-0.73	3.53	-0.02
Judge 1 vs 17	4.51	0.89	5.06	0.02
Judge 1 vs 19	-0.23	-0.05	4.99	-0.00
Judge 1 vs 20	-10.68	-1.09	9.79	-0.02

**Significant at the 1% level.

*Significant at the 5% level.

The interaction between experiment and length of ripening period was tested as shown in Table 8. Although the effect of experiment, length of ripening period, type, judge, and the interaction of experiment and length of ripening period were all significant at the 1% level, all had a small F-ratio except type. This means that the main effect was due to type of cheese. It should be noted that the interaction between the experiment and length of ripening period was used as an error term for length of ripening period and experiment instead of the normal error term.

Table 8. Analysis of variance of flavor scores for experiments 1 to 6

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	1211	1963676.81		
Mean	1	770555.34	770555.34	
Experiment[a]	5	606.49	121.30	5.07**
Length of ripening period[a]	5	315.73	63.15	2.64
Type	9	3147.00	349.67	60.98**
Judge	15	591.33	39.42	6.88**
Experiment x length of ripening period	25	597.97	23.92	4.17**
Error	1151	6599.59	5.73	

[a]F-ratio of experiment and length of ripening period was calculated using the experiment x length of ripening period as an error term.

**Significant at the 1% level.

Table 9 shows the analysis of variance of the interaction between length of ripening period and type. The effect of the interaction was not significant. The effect of experiment, length of ripening period, and type were significant at the 1% level with F-ratios of 20.73, 5.11, 53.74, respectively. The F-ratio for type was much higher than the F-ratios for length of ripening period and experiment.

Comparisons of the pooled flavor scores of the various types of cheese are given in Table 11, and Table 10 presents a key to the type numbers used in Table 11.

The β coefficients from the model used in the analysis of variance were used to predict the flavor scores at the various ripening periods. These corrected flavor responses are plotted in Figures 4-9.

Table 9. Analysis of variance of flavor scores for experiments 1 to 6

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	1222	1981723.67		
Mean	1	1137885.78	1137885.78	
Experiment	5	669.54	133.91	20.73**
Length of ripening period	5	177.98	35.60	5.11**
Type	9	3124.09	347.12	53.74**
Length of ripening period x type	45	376.33	8.36	1.30
Error	1157	7472.92	6.46	

**Significant at the 1% level.

Table 10. Key to the type numbers used in Table 11

Number	Type
1	Milk fat
2	Kaola
3	Kaomel
4	Kaola esterified with tributyrin and tricaprion
5	Mineral oil
6	Mineral oil plus dimethyl sulfide
7	Milk fat plus buttermilk solids
8	Deodorized milk fat
9	Deodorized milk fat with distillate
10	Deodorized milk fat plus dimethyl sulfide

Table 11. Comparison of adjusted means of flavor scores for types of fat for experiments 1 to 6

Comparison of types	Difference in adjusted mean x 10	T-value	Standard error x 10	Standard partial regression coefficient
1 vs 2	28.22	10.79**	2.62	0.32
1 vs 3	63.18	20.78**	3.04	0.62
1 vs 4	25.41	6.02**	4.22	0.17
1 vs 5	14.95	3.35**	4.46	0.11
1 vs 6	15.74	3.53**	4.46	0.11
1 vs 7	13.15	3.26**	4.03	0.09
1 vs 8	5.32	1.70	3.13	0.05
1 vs 9	21.10	6.74**	3.13	0.21
1 vs 10	3.62	0.89	4.05	0.03
2 vs 1	-28.22	-10.79**	2.60	-0.32
2 vs 3	34.96	11.50**	3.04	0.56
2 vs 4	-2.81	-0.67	4.22	-0.04
2 vs 5	-13.27	-2.56*	5.17	-0.19
2 vs 6	-12.47	-2.41*	5.17	-0.18
2 vs 7	-15.07	-3.14**	4.80	-0.21
2 vs 8	-22.90	-5.61**	4.08	-0.37
2 vs 9	-7.12	-1.75	4.08	-0.12
2 vs 10	-24.60	-5.10**	4.83	-0.35
3 vs 1	-63.18	-20.78**	3.04	-0.62
3 vs 2	-34.96	-11.50**	3.04	-0.56
3 vs 4	-37.77	-7.77**	4.86	-0.47
3 vs 5	-48.23	-8.93**	5.40	-0.60
3 vs 6	-47.43	-8.78**	5.40	-0.59
3 vs 7	-50.03	-9.91**	5.05	-0.63
3 vs 8	-57.86	-13.26**	4.36	-0.85
3 vs 9	-42.08	-9.64**	4.36	-0.62
3 vs 10	-59.56	-11.75**	5.07	-0.75
4 vs 1	-25.41	-6.02**	4.22	-0.17
4 vs 2	2.81	0.67	4.22	0.04
4 vs 3	37.77	7.77**	4.86	0.47
4 vs 5	-10.46	-1.70	6.14	-0.11
4 vs 6	-9.66	-1.57	6.14	-0.10
4 vs 7	-12.26	-2.10*	5.84	-0.13
4 vs 8	-20.09	-3.82**	5.26	-0.25
4 vs 9	-4.31	-0.82	5.26	-0.05
4 vs 10	-21.78	-3.72**	5.85	-0.22
5 vs 1	-14.95	-3.35**	4.46	-0.11
5 vs 2	13.27	2.56*	5.17	0.19
5 vs 3	48.23	8.93**	5.40	0.60

**Significant at the 1% level.

*Significant at the 5% level.

Table 11 (Continued)

Comparison of types	Difference in adjusted mean x 10	T-value	Standard error x 10	Standard partial regression coefficient
5 vs 4	10.46	1.70	6.14	0.11
5 vs 6	0.79	0.18	4.45	0.01
5 vs 7	-1.80	-0.30	6.01	-0.02
5 vs 8	-9.63	-1.77	5.45	-0.12
5 vs 9	6.14	1.13	5.45	0.08
5 vs 10	-11.33	-1.88	6.03	-0.12
6 vs 1	-15.74	-3.53**	4.46	-0.11
6 vs 2	12.47	2.41*	5.17	0.18
6 vs 3	47.43	8.78**	5.40	0.59
6 vs 4	9.66	1.57	6.14	0.10
6 vs 5	-0.79	-0.18	4.45	-0.01
6 vs 7	-2.60	-0.43	6.01	-0.03
6 vs 8	-10.42	-1.91	5.45	-0.13
6 vs 9	5.35	0.98	5.45	0.07
6 vs 10	-12.12	-2.01	6.03	-0.13
7 vs 1	-13.15	-3.26**	4.03	-0.09
7 vs 2	15.07	3.14**	4.80	0.21
7 vs 3	50.03	9.91**	5.05	0.63
7 vs 4	12.26	2.10*	5.84	0.13
7 vs 5	1.80	0.30	6.01	0.02
7 vs 6	2.60	0.43	6.01	0.03
7 vs 8	-7.83	-1.94	4.03	-0.10
7 vs 9	7.95	1.97	4.03	0.10
7 vs 10	-9.53	-1.86	5.11	-0.10
8 vs 1	-5.32	-1.70	3.13	-0.05
8 vs 2	22.90	5.61**	4.08	0.37
8 vs 3	57.86	13.26**	4.36	0.85
8 vs 4	20.09	3.82**	5.26	0.25
8 vs 5	9.63	1.77	5.45	0.12
8 vs 6	10.42	1.91	5.45	0.13
8 vs 7	7.83	1.94	4.03	0.10
8 vs 9	15.78	5.04**	3.13	0.23
8 vs 10	-1.70	-0.42	4.05	-0.02
9 vs 1	-21.10	-6.74**	3.13	-0.21
9 vs 2	7.12	1.75	4.08	0.12
9 vs 3	42.08	9.64**	4.36	0.62
9 vs 4	4.31	0.82	5.26	0.05
9 vs 5	-6.14	-1.13	5.45	-0.08
9 vs 6	-5.35	-0.98	5.45	-0.07
9 vs 7	-7.95	-1.97	4.03	-0.10
9 vs 8	-15.78	-5.04**	3.13	-0.23
9 vs 10	-17.47	-4.31**	4.05	-0.22
10 vs 1	-3.62	-0.89	4.05	-0.03
10 vs 2	24.60	5.10**	4.83	0.35
10 vs 3	59.56	11.75**	5.07	0.75
10 vs 4	21.78	3.72**	5.85	0.22
10 vs 5	11.33	1.88	6.03	0.12
10 vs 6	12.12	2.01	6.03	0.13
10 vs 7	9.53	1.86	5.11	0.10
10 vs 8	1.70	0.42	4.05	0.02
10 vs 9	17.47	4.31**	4.05	0.22

Figure 4. Cheddar cheese flavor development during ripening of cheeses made from milk fat, Kaola, and Kaomel (experiment 1)

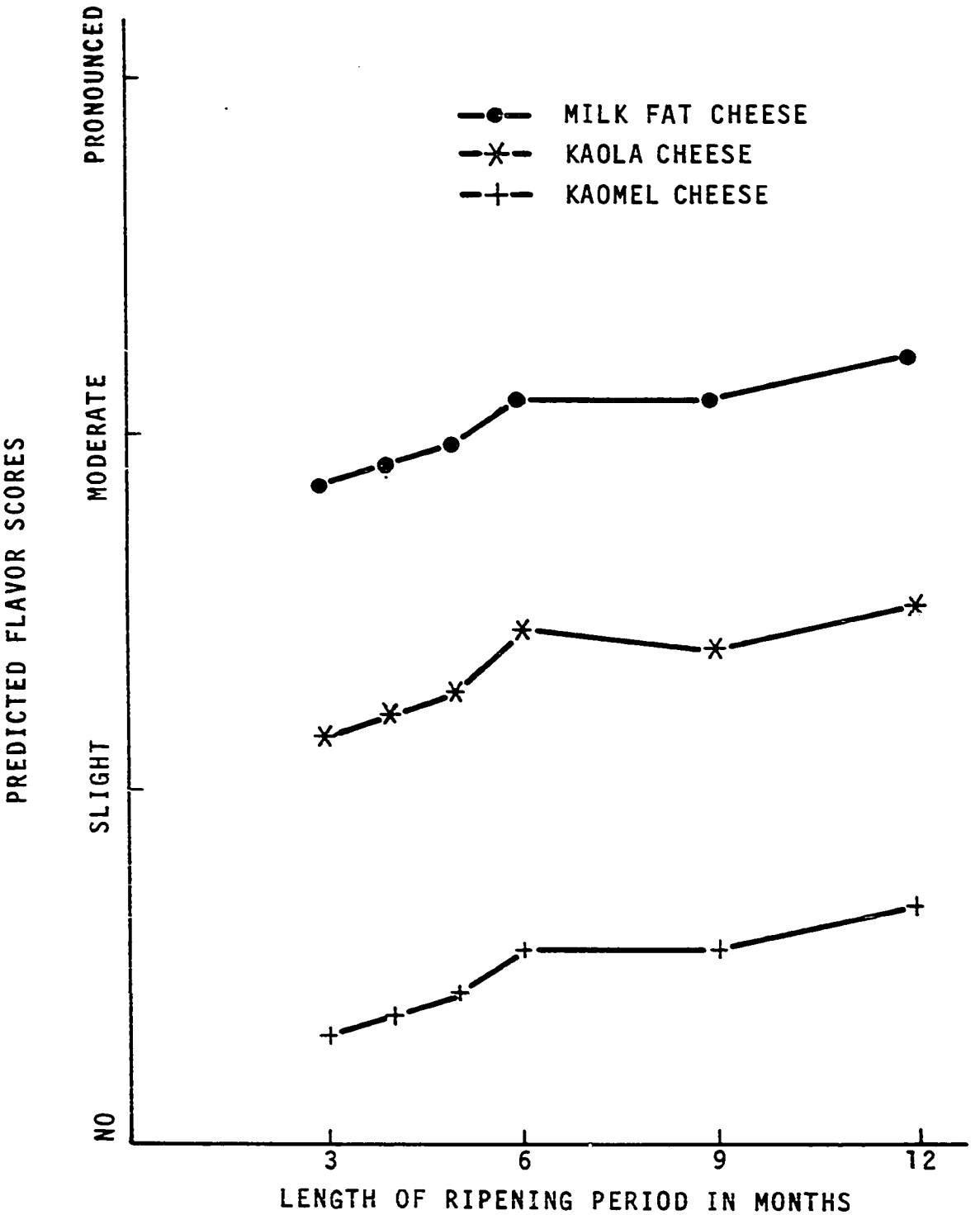

PREDICTED FLAVOR SCORES
PRONOUNCED
MODERATE
SLIGHT
NO
MILK FAT CHEESE
KAOLA CHEESE
KAOMEL CHEESE
3
6
9
12
LENGTH OF RIPENING PERIOD IN MONTHS

Figure 5. Cheddar cheese flavor development during ripening of cheeses made from milk fat, Kaola, and Kaomel (experiment 2)

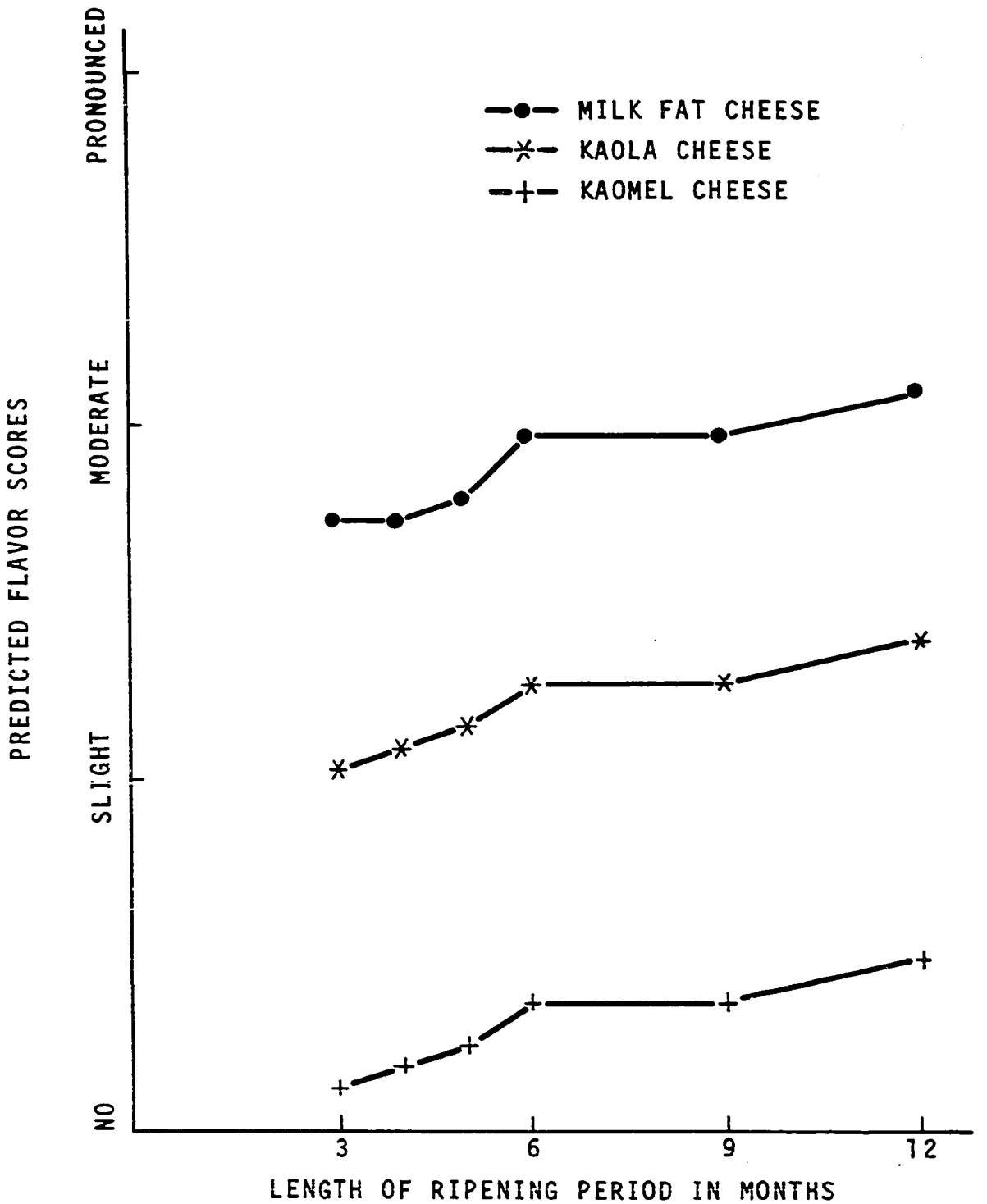

PREDICTED FLAVOR SCORES
PRONOUNCED
MODERATE
SLIGHT
NO
MILK FAT CHEESE
KAOLA CHEESE
KAOMEL CHEESE
3
6
9
12
LENGTH OF RIPENING PERIOD IN MONTHS

Figure 6. Cheddar cheese flavor development during ripening of cheeses made from milk fat, milk fat plus buttermilk solids, deodorized milk fat, and deodorized milk fat plus proportional amount of distillate (experiment 3)

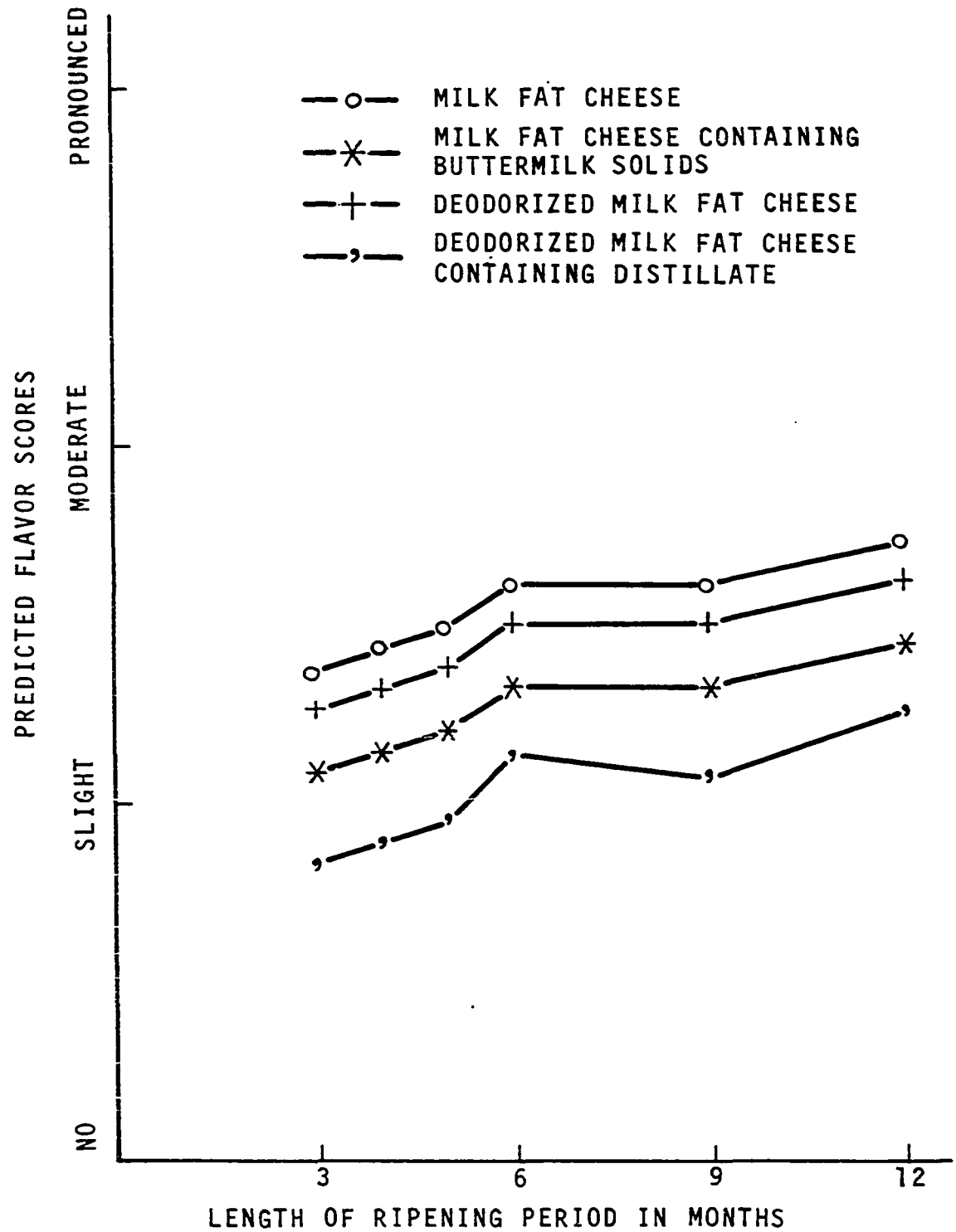

MILK FAT CHEESE
MILK FAT CHEESE CONTAINING BUTTERMILK SOLIDS
DEODORIZED MILK FAT CHEESE
DEODORIZED MILK FAT CHEESE CONTAINING DISTILLATE
PREDICTED FLAVOR SCORES
PRONOUNCED
MODERATE
SLIGHT
NO
3
6
9
12
LENGTH OF RIPENING PERIOD IN MONTHS

Figure 7. Cheddar cheese flavor development during ripening of cheeses made from milk fat, Kaola, and esterified Kaola (experiment 4)

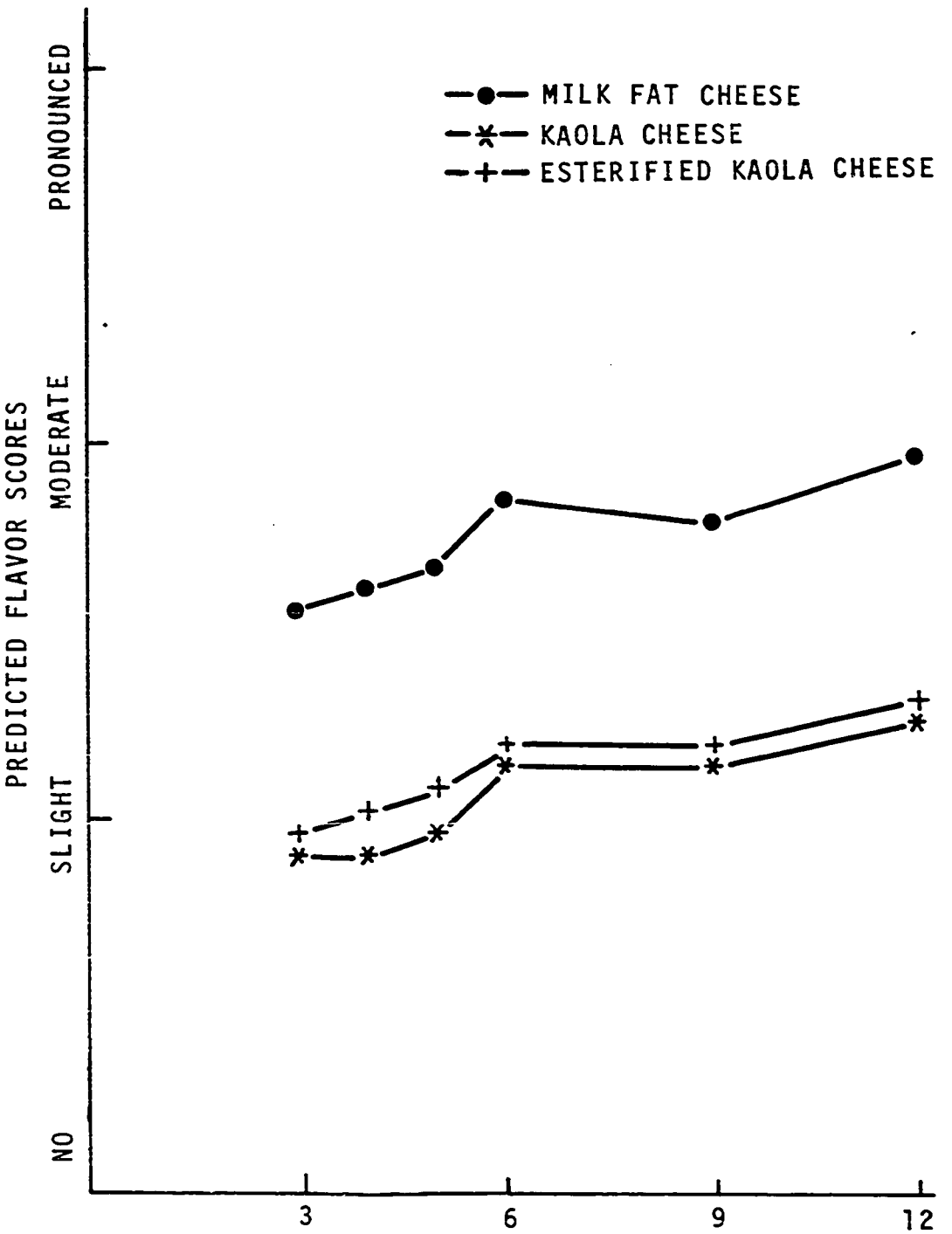

MILK FAT CHEESE
KAOLA CHEESE
ESTERIFIED KAOLA CHEESE
PREDICTED FLAVOR SCORES
PRONOUNCED
MODERATE
SLIGHT
NO
3
6
9
12

Figure 8. Cheddar cheese flavor development during ripening of cheeses made from milk fat, mineral oil, and mineral oil containing 40 ppm dimethyl sulfide (experiment 5)

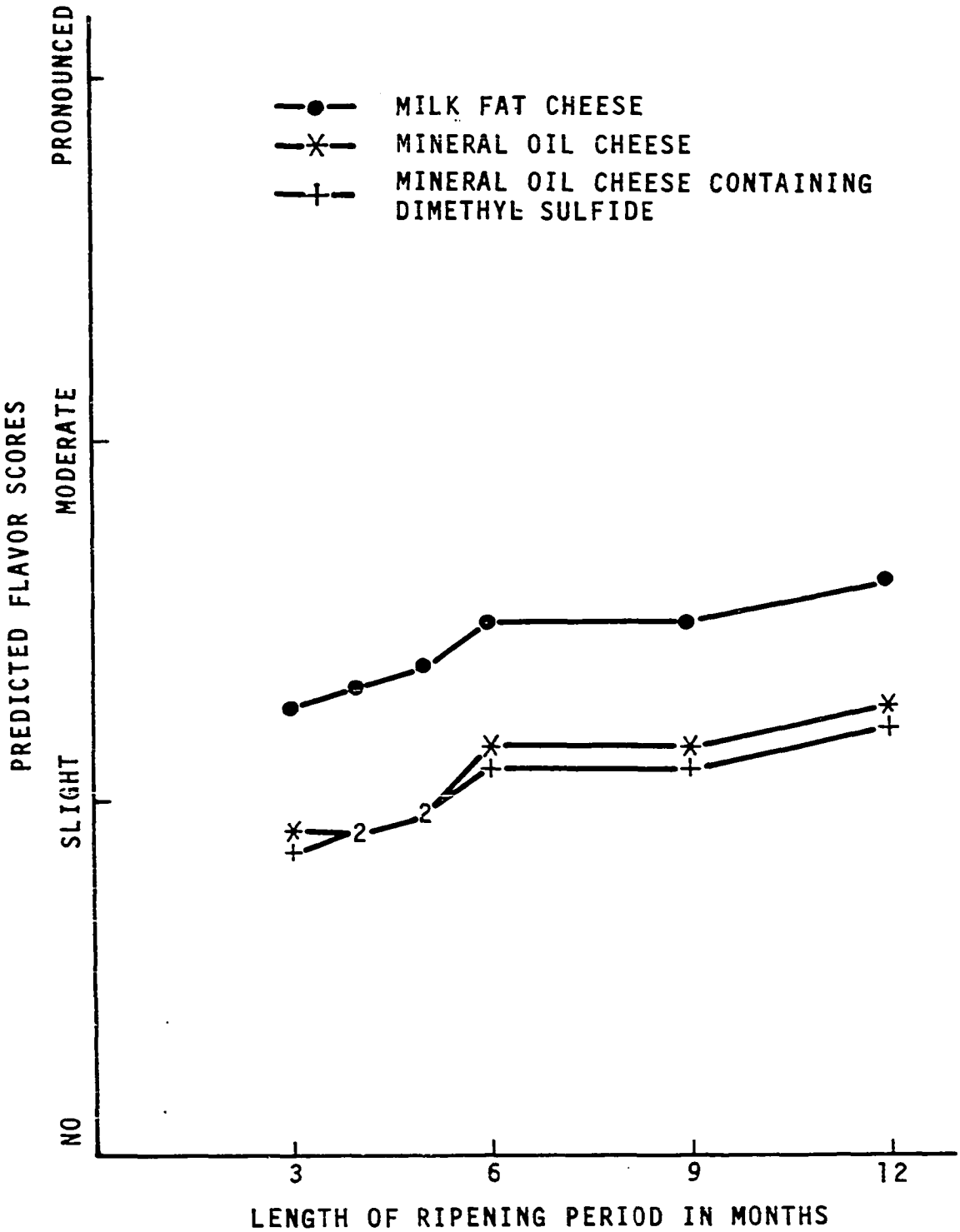
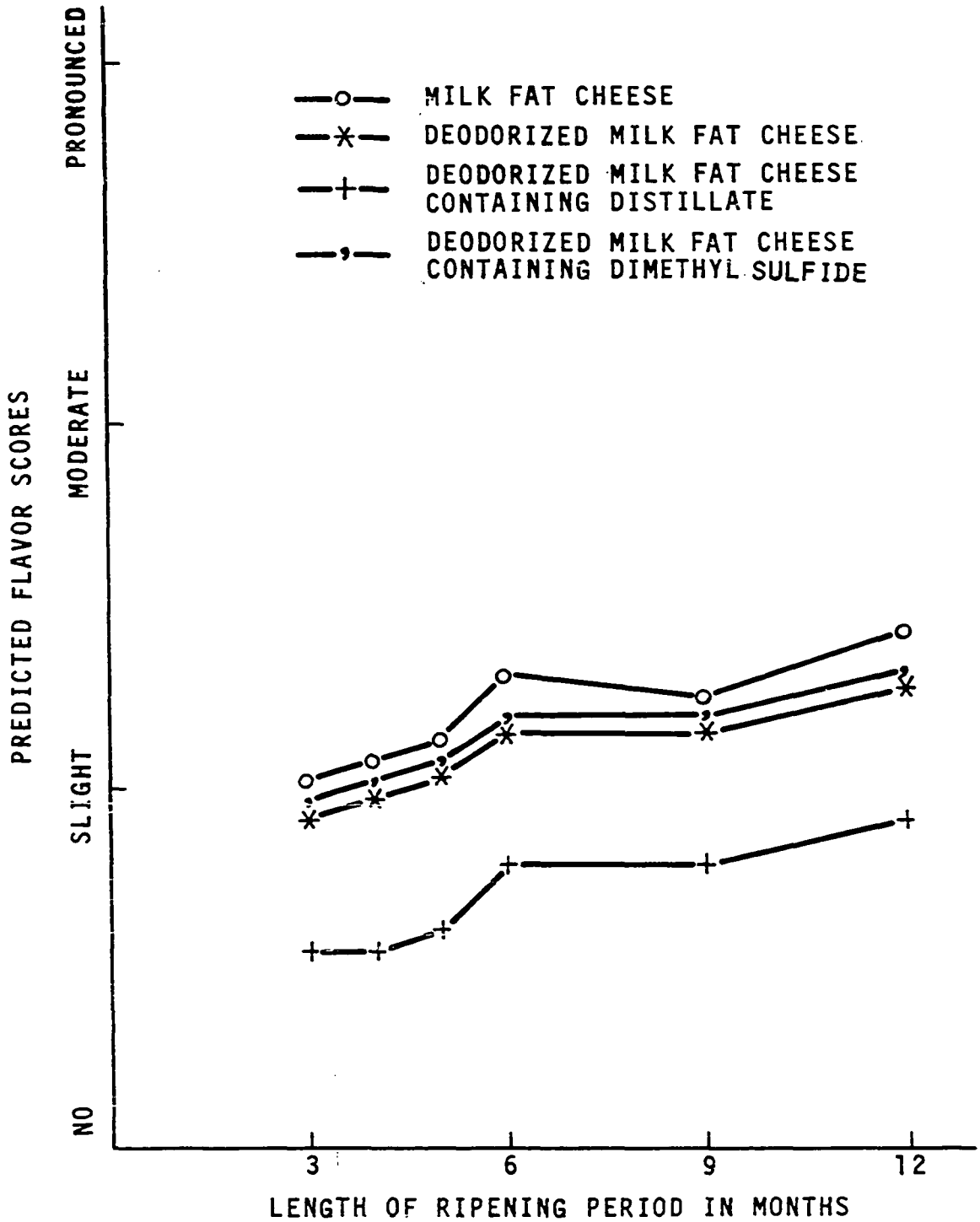

PRONOUNCED
MODERATE
SLIGHT
NO
PREDICTED FLAVOR SCORES
MILK FAT CHEESE
MINERAL OIL CHEESE
MINERAL OIL CHEESE CONTAINING DIMETHYL SULFIDE
2
2
3
6
9
12
LENGTH OF RIPENING PERIOD IN MONTHS

Figure 9. Cheddar cheese flavor development during ripening of cheeses made from milk fat, deodorized milk fat, deodorized milk fat containing 40 ppm dimethyl sulfide, and deodorized milk fat containing a proportionate amount of distillate (experiment 6)


MILK FAT CHEESE
DEODORIZED MILK FAT CHEESE
DEODORIZED MILK FAT CHEESE CONTAINING DISTILLATE
DEODORIZED MILK FAT CHEESE CONTAINING DIMETHYL SULFIDE
PREDICTED FLAVOR SCORES
PRONOUNCED
MODERATE
SLIGHT
NO
3
6
9
12
LENGTH OF RIPENING PERIOD IN MONTHS

Effect of ripening period

Table 6 and Figures 4 to 9 show that the cheese had developed more flavor as it matured, and this was true for all the experiments. Table 6 shows that there was no significant difference between 3 months and 4 or 5 months. The difference in flavor score between 3 months and 6, 9, or 12 months was significant at the level of 1%. Also, it is clear that the cheeses improved in flavor during ripening, especially up to 6 months, after which the change in flavor was minimal. Figures 4 to 9 show that most of the predicted flavor score for these cheeses were "moderate" or lower on the scale at the end of the ripening period. It should be indicated that none of these cheeses had developed a full flavor of Cheddar cheese as none of the predicted flavor scores was "pronounced" on the scale, "pronounced" resembling a full flavor of Cheddar cheese.

Type comparison

Table 11 and Figures 4 to 9 show that milk fat cheese, which was used as a control, was the best in Cheddar cheese flavor development in relation to the experimental cheeses. The predicted flavor score for milk fat was either "moderate" or less on the scale which indicates that the milk fat cheese had not developed a full flavor of Cheddar cheese. Later a comparison between milk fat cheese and conventional cheese was made (Table 12) to show the analysis of variance of flavor score for milk fat cheese and conventional cheese. The difference between these two cheeses was significant at the level of 1%.

The types of fat used in experiments 1 and 2 were milk fat (type 1), Kaola (type 2), and Kaomel (type 3). Kaola and Kaomel are recommended

as milk fat substitutes in various cheeses. The fatty acid profile of butter fat, Kaola, and Kaomel were reported by Ryberg (79) and are shown in Table 21 in the Appendix.

The material labeled as Kaola has levels of short chain fatty acid approximately the same as milk fat. It is recommended that this oil be used in conjunction with a low level of lipolyzed milk fat. It is supposed to provide a Cheddar flavor equivalent to milk fat. The other oil, Kaomel, is recommended on the basis of physical properties, complete lack of short chain fatty acids, its great stability, and its blandness. It is supposed to be bland even in the presence of lipase activity.

Table 21 in the Appendix shows that Kaola (type 2) does not contain butyric acid and is low in caproic acid. Therefore, experiment 4 was conducted using milk fat and Kaola and Kaola with added tributyrin and tricaprion to bring the levels of these fatty acids up to the levels found in milk fat.

Figures 4 and 5 present the predicted flavor scores of milk fat, Kaola, and Kaomel cheeses made in experiments 1 and 2. Milk fat cheese shows a higher predicted flavor score than Kaola or Kaomel cheeses during the entire ripening period.

Table 11 shows that the difference comparing Kaola (type 2) cheese with esterified Kaola (type 4) cheese was not significant. The Kaola (type 2) cheese was better and significantly different than Kaomel (type 3) cheese at the level of 1% with a t-value of 11.50. There was no significant difference between Kaola (type 3) and esterified Kaola (type 4) or deodorized milk fat containing porportional amount of distillate

(type 9). The Kaola (type 2) cheese was significantly lower than mineral oil (type 5) cheese and mineral oil containing 40 ppm dimethyl sulfide (type 6) cheese at the 5% level with a t-value of 2.56 and 2.41, respectively. Also, it was lower than milk fat cheese (type 1) and milk fat containing buttermilk solids (type 7), deodorized milk fat (type 8), and deodorized milk fat cheese containing dimethyl sulfide (type 10) at the 1% level with t-values of 10.79, 3.14, 5.61, and 5.10, respectively. Kaomel cheese was significantly lower than the other nine cheeses at the level of 1%. It is clear from Table 11 and Figures 4 and 5 that the Kaola cheese (type 2) was lower in the predicted flavor score than milk fat cheese (type 1), and Kaomel cheese (type 3) was even worse. This indicates that the vegetable oils, i.e., Kaola and Kaomel, either do not contain the precursors of Cheddar cheese flavor or developed off flavors which masked the Cheddar flavor.

The addition of short chain fatty acids, as in experiment 4, did not greatly improve the flavor. Table 11 and Figure 7 show that the esterification of butyric acid and caproic acid in Kaola slightly improved the predicted flavor scores, but they were still much lower than the milk fat cheese scores.

Table 11 shows that there was no significant difference between cheese made from esterified Kaola (type 4) and Kaola (type 2) or mineral oil (type 5) or mineral oil containing 40 ppm dimethyl sulfide (type 6) or deodorized milk fat containing a proportional amount of distillate (type 9). Esterified Kaola (type 4) was significantly lower at the level of 1% than milk fat containing buttermilk solids (type 7) cheese with a t-value of 2.10. Also, esterified Kaola (type 4) was significantly

lower at the level of 1% than milk fat cheese (type 1), deodorized milk fat cheese (type 8), or deodorized milk fat containing 40 ppm dimethyl sulfide cheese (type 10), with t-values of 6.02, 3.82, and 3.72, respectively. There was no significant difference between Kaola cheese (type 2) and esterified Kaola cheese (type 4). This indicates that the addition of butyric and caproic did not improve the flavor greatly. Liebich et al. (54) suggested that free fatty acids are the basis for any cheese flavor; however, characteristic aroma for each type of cheese depends on the proportion of the free fatty acids and other volatile components.

Mabbitt and Zielinska (58) and Ohren and Tuckey (66) reported that milk fat appeared to be essential for the production of typical Cheddar flavor. When Cheddar cheese was made with skim milk, the resultant cheese after ripening had a broth-like flavor. Further, Ohren and Tuckey added that only Cheddar cheese containing 50% fat or more on the basis of the dry matter developed a typical Cheddar cheese flavor.

In contrast, Table 11 shows that mineral oil cheese (type 5) developed a Cheddar flavor significantly higher than Kaola cheese (type 2) at the level of 5% with a t-value of 2.56, and higher than Kaomel cheese (type 3) at the level of 1% with a t-value of 8.93. However, mineral oil cheese (type 5) was lower in the predicted flavor score than milk fat cheese (type 1) as shown in Figure 8. The difference was significant at the level of 1% with a t-value of 3.35 as shown in Table 11. There was no significant difference between mineral oil cheese (type 5) and the rest of the types. Although mineral oil cheese (type 5) did not develop an off flavor, it did not develop as good a flavor as milk

fat cheese (type 1) as shown in Figure 8. This lack of flavor development might be due to the lack of short chain fatty acids. The mineral oil cheese (type 5) developed more flavor than we expected. This might support the theory that fat in cheese is just a solvent media for the components of Cheddar cheese flavor. The fat or the oil might trap the flavor and concentrate it in the fat or oil media. The fat or oil might preserve the flavor so it can stay strong and potent.

It is believed that lactone and ketones are important in some milk fat flavor and Cheddar cheese (O'Keefe et al., 68; Liebich et al., 54). Deodorized milk fat cheese (type 8) and deodorized milk fat with a proportionate amount of distillate (type 9) had been used for making cheese as in experiments 3 and 6. The milk fat deodorization process was carried out according to Dimick and Walker (20). By using a steam distillation the β-keto acids and hydroxy acids could be hydrolyzed and removed with the steam as reported by Kinsella et al. (37) and shown in Figures 10 and 11.

Table 11 shows no significant difference between deodorized milk fat cheese (type 8) and milk fat cheese (type 1), mineral oil cheese (type 5), mineral oil plus dimethyl sulfide cheese (type 6), milk fat plus buttermilk solids (type 7), or deodorized milk fat plus 40 ppm dimethyl sulfide cheese (type 10). Deodorized milk fat cheese (type 8) was significantly higher at the level of 1% in flavor score when compared to Kaola cheese (type 2), Kaomel cheese (type 3), esterified Kaola cheese (type 4), and deodorized milk fat with proportionate distillate (type 9), with t-values of 5.61, 13.26, 3.82, and 5.04, respectively.

Figure 10. Mechanism of formation of methyl ketone

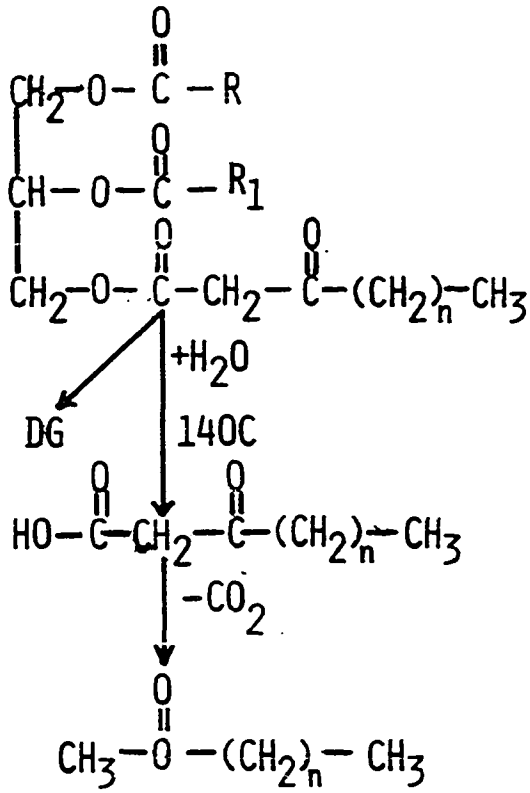
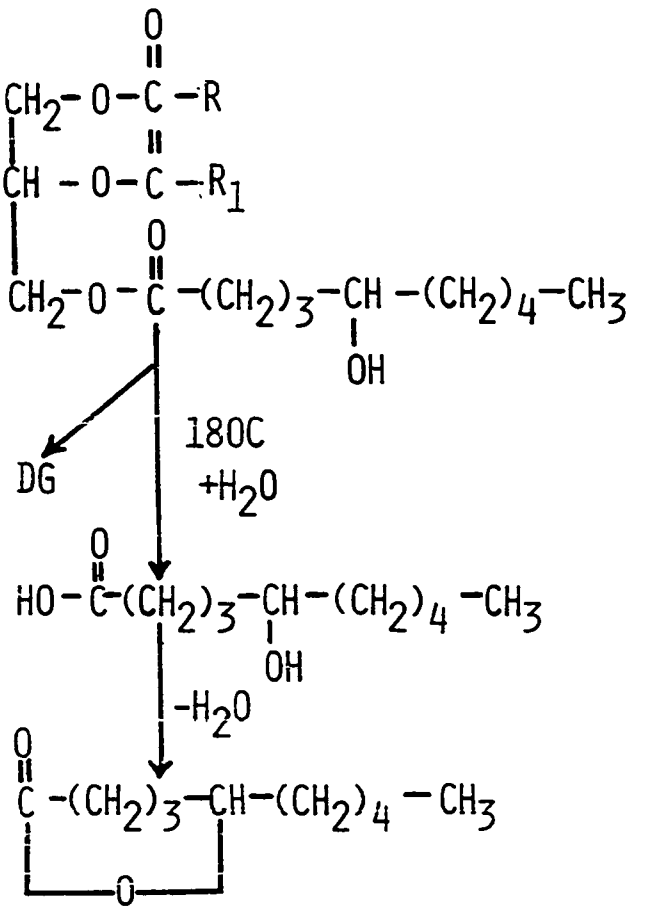

CH2-O-C-R
CH-O-C-R1
CH2-O-C-CH2-C-(CH2)n-CH3
+H2O
DG
140C
HO-C-CH2-C-(CH2)n-CH3
-CO2
CH3-O-(CH2)n-CH3

Figure 11. Mechanism of lactone formation


O
CH2-O-C-R
CH-O-C-R1
O
CH2-O-C-(CH2)3-CH-(CH2)4-CH3
OH
DG
180C
+H2O
O
HO-C-(CH2)3-CH-(CH2)4-CH3
OH
-H2O
O
C-(CH2)3-CH-(CH2)4-CH3
O

The addition of distillate, which should contain lactones and methyl ketones, to the deodorized milk fat (type 9) lowered the flavor scores greatly as shown in Figures 6 and 9. Deodorized milk fat containing a proportionate amount of distillate (type 9) was significantly lower than milk fat cheese (type 1) or deodorized milk fat cheese (type 8) or deodorized milk fat containing 40 ppm dimethyl sulfide cheese (type 10) at the level of 1% with t-values of 6.74, 5.04, and 4.31, respectively. This means that the addition of the distillate, i.e., lactones and methyl ketones, did not improve the flavor as claimed by many workers (Kroger and Patton, 50; Day and Libbey, 19; Morris et al., 64; O'Keefe et al., 68; Liebich et al., 54).

It was reported that volatile sulfur compounds are produced in Cheddar cheese and may contribute to the flavor of Cheddar cheese (Kristoffersen and Nelson, 49; Kristoffersen and Gould, 43; Patton et al., 73; Walker, 94; Walker and Harvey, 96). Dimethyl sulfide was added in a concentration of 40 ppm to mineral oil and to deodorized milk fat, and was possibly removed during deodorization. Table 11 shows the comparison of cheese containing mineral oil plus 40 ppm dimethyl sulfide (type 6) with the other types. There was no significant difference between cheese with mineral oil containing 40 ppm dimethyl sulfide (type 6) and mineral oil cheese (type 5). The cheese with mineral oil plus 40 ppm dimethyl sulfide (type 6) was significantly lower at the level of 1% than the milk fat cheese (type 1) with a t-value of 3.53, as shown in Table 11 and Figure 8.

The addition of dimethyl to the deodorized milk fat appeared to improve the flavor as there was no significant difference between milk

fat cheese (type 1) and cheese with deodorized milk fat plus 40 ppm dimethyl sulfide (type 10). Figure 9 shows cheese with deodorized milk fat plus 40 ppm dimethyl sulfide was slightly better than deodorized milk fat cheese, although the difference was not significant. The addition of dimethyl sulfide apparently improved the flavor with milk fat better than with mineral oil as shown in Figures 8 and 9, respectively. However, this cannot be used to support the importance of the sulfur compounds in Cheddar cheese flavor as stated by earlier workers.

Comparison of milk fat cheese and conventional cheese

The cheese made by homogenizing milk fat into skim milk gave flavor scores indicating at best a moderate intensity of Cheddar flavor. To investigate this further we compared some milk fat cheese with conventional Cheddar of the same age. The age of the cheese ranged from 6 to 12 months when the comparisons were made. The crude average flavor scores for conventional cheese and milk fat cheese at the age of 12 months were 10.6 and 6.70, respectively. Table 12 presents the analysis of variance of flavor scores for testing milk fat cheese against conventional cheese.

Table 12. Analysis of variance of flavor scores for testing milk fat cheese against conventional cheese

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	127	1556.70		
Regression	7	353.11	50.44	
Milk fat cheese x conventional cheese	1	135.71	135.71	13.53**
(Experiment-group) (type)	6	217.40	36.23	3.61**
Error	120	1203.58	10.03	

**Significant at the 1% level.

Notice in Table 12 that the difference between conventional cheese (type 11) and milk fat cheese (type 1) was significant at the 1% level with an F-ratio of 13.53. Although the effect of (experiment-group) (type) was significant at the 1% level, the F-ratio of 3.61 was relatively small as compared to the effect of type or the difference between conventional cheese and milk fat cheese.

The only difference in constituents between conventional cheese and milk fat cheese was fat globule membrane and the enzymes attached to it. The fat globule membrane and the enzymes are washed out in the preparation of milk fat.

Buttermilk solids which contain some milk fat globule membrane material were added to the cheese milk. Table 11 presents the comparisons of cheese made with milk fat plus buttermilk solids (type 7) and other types of cheeses. It is clear that the addition of buttermilk solids did not improve the flavor as shown in Figure 6, since the cheese containing buttermilk solids gave scores significantly lower than those of the milk fat cheese. This is probably because the buttermilk solids were not bland enough and gave an objectionable flavor to the resultant cheese.

Next we decided to prepare milk fat globule membrane material from washed cream by a freeze drying technique so that the solids content of the cheese and the flavor of the membrane material could be better controlled and the enzyme activity be better preserved.

Experiment 7 was conducted to test the effect of the fat globule membranes and their enzymes on cheese flavor. Four treatments were used: milk fat plus heated fat globule membrane, milk fat plus fat globule

membrane, mineral oil plus heated fat globule membrane, and mineral oil plus fat globule membrane. Heated fat globule membrane should have no enzyme activity. The cheese was tasted after 3, 4, 5, 6, and 9 months ripening. Table 13 shows the analysis of variance of the flavor scores.

Table 13. Analysis of variance of flavor scores for experiment 7

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	188	289298.23		
Mean	1	255462.96	255462.96	
Length of ripening period	4	117.69	29.42	5.16**
Type	3	12.82	4.27	0.75
Judge	12	279.65	23.30	4.09**
Length of ripening period x type	12	90.30	7.52	1.32
Error	156	889.12	5.70	

**Significant at the 1% level.

The effects of type and interaction of length of ripening period and type were not significant at the 5% level with F-ratios of 0.75 and 1.32, respectively. The effects of length of ripening period and judge were significant at the 1% level with F-ratios of 5.16 and 4.09, respectively. The results indicate there was no difference between milk fat cheese and mineral oil cheese, although Table 11 shows a significant difference at the 1% level between milk fat cheese and mineral oil cheese. This might be caused by the addition of fat globule membrane material or to the insufficient observations in experiment 7. Also, this analysis showed no difference between the fat globule membrane and heated fat globule membrane. One may conclude that fat globule membrane enzymes destroyed as a result of

this heat treatment were not important in developing Cheddar cheese flavor. The fat globule membrane was slightly oxidized due to the length of time it took to get it freeze dried with the facilities available. The fat globule membrane flavor might affect the ability of the judges in scoring these cheeses. Since only four sorts of cheese could be made in an experiment no milk fat control was made. The crude (average) flavor scores are plotted in Figure 12. These indicate the flavor was about the same as milk fat cheese in the other experiments.

Next we considered the possibility that conventional cheese has a better flavor than milk fat cheese because the membrane material serves as an emulsifying agent. This effect might not be duplicated by adding back freeze-dried membrane to milk fat cheese.

Experiment 8 was conducted to test the effect of gum acacia on stabilizing the homogenized fat. The flavor was examined after 3, 4, 5, and 6 months ripening. Table 14 presents the analysis of variance of flavor scores for experiment 8.

The effect of length of ripening period was not significant; however, the effects of judge, type, and the interaction between length of ripening period and type were significant at the 1% level with F-ratios of 3.38, 27.08, and 4.77, respectively. The gum acacia raised the mean flavor scores to 8.79, 9.03, 8.62, and 8.51 compared with blank (milk fat) of 3.93, 4.23, 5.84, and 7.48 at ripening periods of 3, 4, 5, and 6 months, respectively.

Figure 12. Cheddar cheese flavor development during ripening of cheeses made from milk fat plus fat globule membrane, milk fat plus heated fat globule membrane, mineral oil plus fat globule membrane, and mineral oil plus heated fat globule membrane

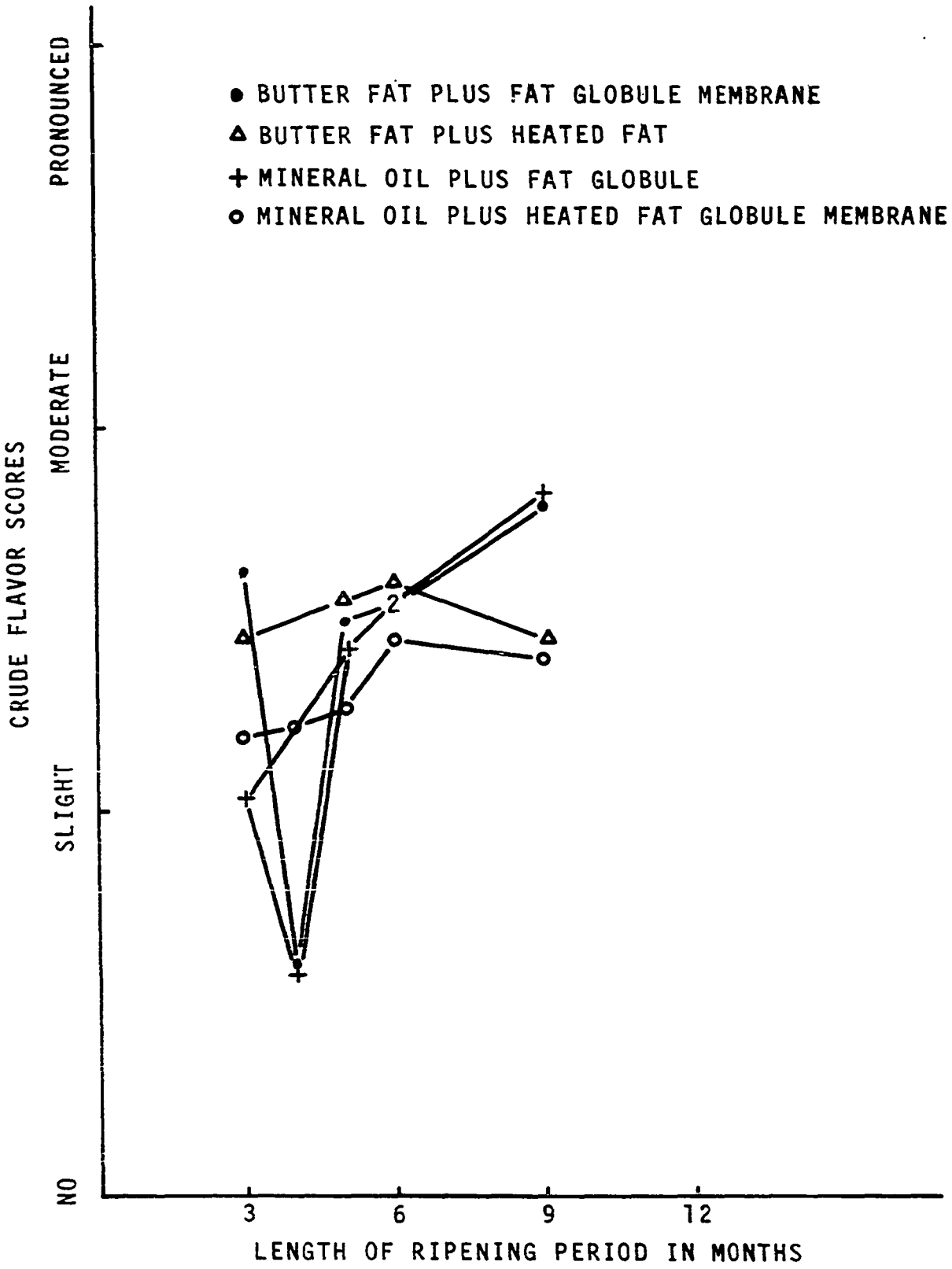

● BUTTER FAT PLUS FAT GLOBULE MEMBRANE
△ BUTTER FAT PLUS HEATED FAT
+ MINERAL OIL PLUS FAT GLOBULE
o MINERAL OIL PLUS HEATED FAT GLOBULE MEMBRANE
CRUDE FLAVOR SCORES
PRONOUNCED
MODERATE
SLIGHT
NO
3
6
9
12
LENGTH OF RIPENING PERIOD IN MONTHS

Table 14. Analysis of variance of flavor scores for experiment 8

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	80	114687.75		
Mean	1	84815.33	84815.33	
Length of ripening period	3	9.50	3.17	0.53
Type	1	160.49	160.49	27.08**
Judge	12	240.55	20.05	3.38**
Length of ripening period x type	3	84.84	28.28	4.77**
Error	60	355.59	5.93	

**Significant at the 1% level.

Comparison of flavor score with proteolysis

Some judges reported a difference in texture between milk fat cheese and conventional cheese, so proteolysis determinations were made on cheese 12 months old. The data are presented in Table 15.

Table 15. Cheese proteolysis and the average of flavor scores at 12 months old

Type of fat	Type no.	Proteolysis	Average flavor score
Experiment 1			
Milk fat	0	23.31 24.07	7.45
Kaola	1	23.93 23.00	5.29
Kaomel	2	22.33 22.26	2.54
Conventional cheese	10	27.44 28.48	--
Experiment 2			
Milk fat	0	23.62 23.31	8.36
Kaola	1	23.44 22.78	5.97

Table 15 (Continued)

Type of fat	Type no.	Proteolysis	Average flavor score
Kaomel	2	22.72 22.49	2.16
Experiment 3			
Milk fat	0	28.17 29.91	6.69
Milk fat plus buttermilk solids	6	24.42 27.18	5.56
Deodorized milk fat	7	26.76 26.48	5.72
Deodorized milk fat plus distillate	8	28.49 28.33	4.89
Conventional cheese	10	28.15 27.17	--
Experiment 4			
Milk fat	0	28.14 27.11	6.16
Kaola	1	25.68 26.43	3.30
Esterified Kaola	3	27.51 27.23	3.64
Conventional cheese	10	27.88 28.28	--
Experiment 5			
Milk fat	0	22.00 23.05	7.45
Mineral oil	4	22.53 22.99	4.82
Mineral oil, 40 ppm dimethyl sulfide/g	5	22.52 23.23	3.92
Conventional cheese	10	29.06 30.16	--
Experiment 6			
Milk fat	0	23.14 24.13	5.29
Deodorized milk fat	7	23.65 23.39	5.60
Deodorized milk fat, 40 ppm dimethyl sulfide/g	9	21.39 22.43	5.48
Deodorized milk fat plus distillate	8	23.21 23.45	3.50
Conventional cheese	10	28.18 28.84	--

Table 16 presents the analysis of variance of proteolysis for testing cheese with various modified types of fat against conventional cheese for experiments 1 to 6.

Table 16. Analysis of variance of proteolysis for testing 1 to 10 fat types of cheese against conventional cheese for experiments 1 to 6

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total (corrected)	24	156.77		
Types	10	96.31	9.63	2.23
Error	14	60.46	4.32	

Table 16 indicates that there was no significant difference in proteolysis between the cheese with modified fat and conventional cheese. This could be because the conventional cheese used for these comparisons was not made from the same milk as the other cheeses with modified fats. Even though the F-test was not quite significant at the 5% level, a t-test was conducted to test the difference between conventional cheese and each of the types of cheese in experiments 1 to 6. The results are shown in Table 17.

On the extent of proteolysis basis, as shown in Table 17, there were no significant differences between conventional cheese and esterified Kaola cheese (type 4), milk fat plus buttermilk solids cheese (type 7), deodorized milk fat cheese (type 8), or deodorized milk fat plus a proportionate amount of distillate (type 9). On the other hand, there were significant differences at the 5% level between conventional cheese and milk fat cheese (type 1), Kaola cheese (type 2), mineral oil cheese

Table 17. Comparison of proteolysis for 1 to 10 types of fat cheese against conventional cheese for experiments 1 to 6

Comparison[a]	Difference in adjusted mean x 10	T-value	Standard error x 10	Standard partial regression coefficient
Conventional cheese vs type 1	-3.36	-2.67*	1.26	-0.57
Conventional cheese vs type 2	-4.15	-2.73*	1.52	-0.54
Conventional cheese vs type 3	-5.91	-3.40**	1.74	-0.64
Conventional cheese vs type 4	-0.99	-0.44	2.28	-0.08
Conventional cheese vs type 5	-5.60	-2.46*	2.28	-0.44
Conventional cheese vs type 6	-5.48	-2.41*	2.28	-0.43
Conventional cheese vs type 7	-2.56	-1.13	2.28	-0.20
Conventional cheese vs type 8	-3.29	-1.89	1.74	-0.36
Conventional cheese vs type 9	-2.49	-1.43	1.74	-0.27
Conventional cheese vs type 10	-6.45	-2.84*	2.28	-0.51

[a]See Table 10 for a key to the cheese types.

**Significant at the 1% level.

*Significant at the 5% level.

(type 5), mineral oil plus 40 ppm dimethyl sulfide (type 6), and deodorized milk fat plus 40 ppm dimethyl sulfide (type 10). The difference in proteolysis between conventional cheese and Kaomel cheese (type 3) was significant at the 1% level. This may account for the hardness and the crumbliness quality of the Kaomel cheese even at the end of the 12-month ripening period.

Table 18 shows the analysis of variance for flavor scores regressed on the extent of proteolysis for experiments 1 to 6 with a small multiple R square of 0.29. Table 19 shows the analysis of variance for the extent of proteolysis regressed on flavor scores for experiments 1 to 6 with a large multiple R square of 0.92.

Table 18. Analysis of variance for flavor scores regressed on proteolysis for experiments 1 to 6

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	25	66.41		
Regression	8	19.24	2.41	0.867
Error	17	47.16	2.77	

Multiple R square = 0.29

Table 19. Analysis of variance for proteolysis regressed on flavor scores for experiments 1 to 6

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Total	25	184.67		
Regression	8	169.07	21.13	23.02
Error	17	15.61	0.92	

Multiple R square = 0.92

The differences in multiple R square in Tables 18 and 19 are due to the fact that the flavor score had a wide range in all the experiments, but the proteolysis had a narrow range distinct for each experiment. The relation of flavor score and the extent of proteolysis is not statistically significant, which indicates that one cannot be reliably predicted from the other. The calculated partial correlation between flavor and proteolysis within experiment was not significant. This means that the experiment effect was great in calculating multiple R square.

Organoleptic Analysis of Cheddar Cheese Volatiles

The foregoing results clearly show that Cheddar cheese made with different kinds of fats had different flavors; however, it was impossible to say what caused these differences when the cheese was examined directly. We hoped that by isolating the cheese fat from different types of cheese, preparing a flavor concentrate by distillation of the fat, and resolution of the flavor compounds by gas chromatography, the differences among cheese types might be more obvious.

The retention times of the aromas from various types of cheese on apeizon L and butanediol succinate columns are given in Figures 13 to 24. The retention times of a series of aliphatic acids and ketones chromatographed under the same conditions are given as a comparison. These cheeses were 12-months old except in Figure 17 which shows the volatiles of a conventional Cheddar cheese of 1-day old.

Figures 13 to 22 show a tendency for certain dominant flavors to appear in all samples; however, there is great individual difference in type and strength of aroma from sample to sample. The differences in samples of the same type were, unfortunately, as great as those between different types.

Terminology was a problem. For instance, the terms vinegary, spicy, acetic acid, and pickles probably mean the same thing. The panelists used one term one time and another term the next time.

The retention time of these flavors varied a little. This variation in retention time may be due to the injection, heating the stainless steel U, or the recording.

Figure 13. Organoleptic analysis of conventional Cheddar cheese volatiles separated on a 30% apeizon L column

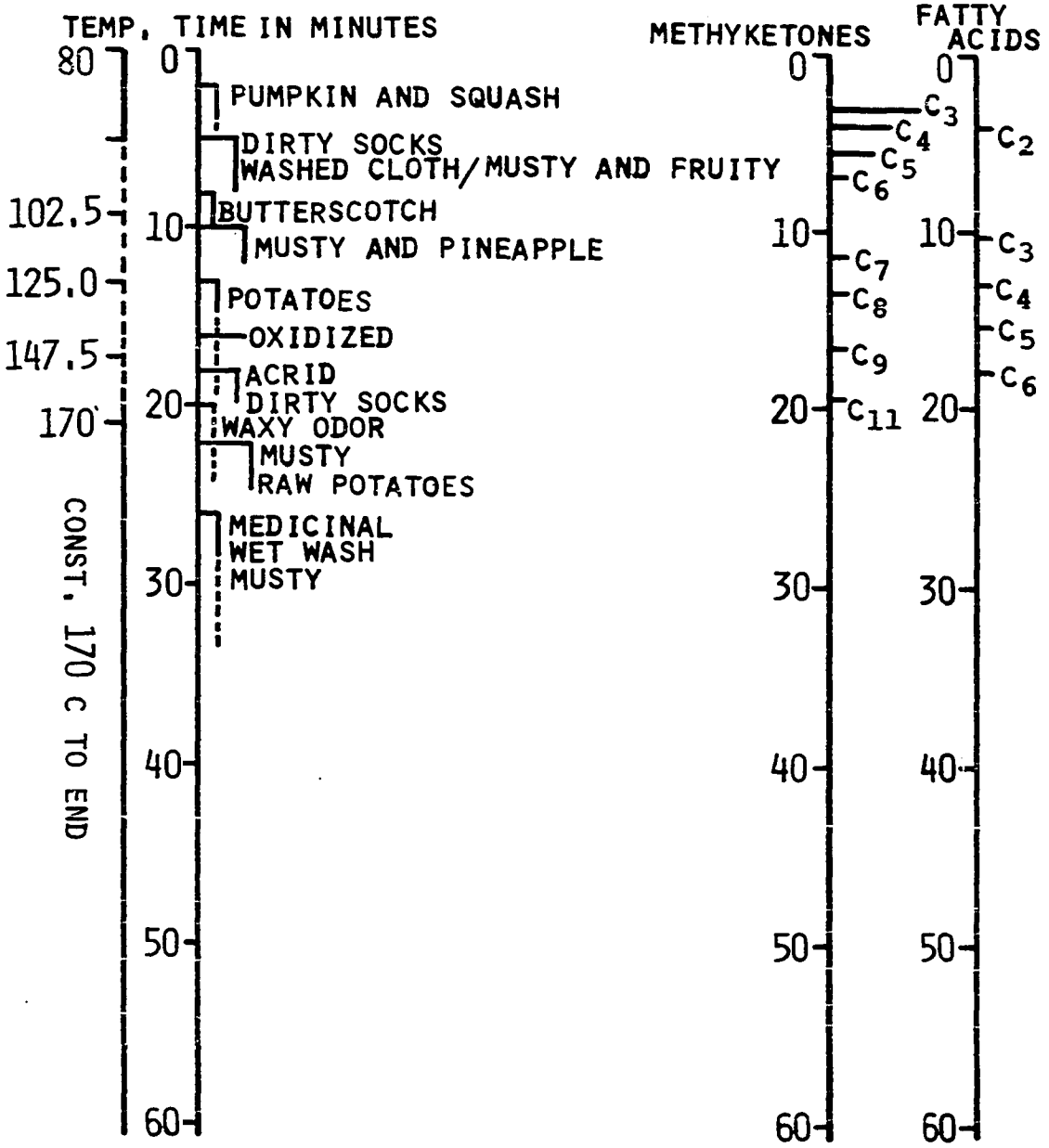

TEMP.
TIME IN MINUTES
METHYKETONES
FATTY ACIDS
80
102.5
125.0
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
PUMPKIN AND SQUASH
DIRTY SOCKS
WASHED CLOTH/MUSTY AND FRUITY
BUTTERSCOTCH
MUSTY AND PINEAPPLE
POTATOES
OXIDIZED
ACRID
DIRTY SOCKS
WAXY ODOR
MUSTY
RAW POTATOES
MEDICINAL
WET WASH
MUSTY
C_3
C_4
C_5
C_6
C_7
C_8
C_9
C_{11}
C_2
C_3
C_4
C_5
C_6

Figure 14. Organoleptic analysis of Kaola cheese volatiles from experiment 1 separated on a 30% apeizon L column

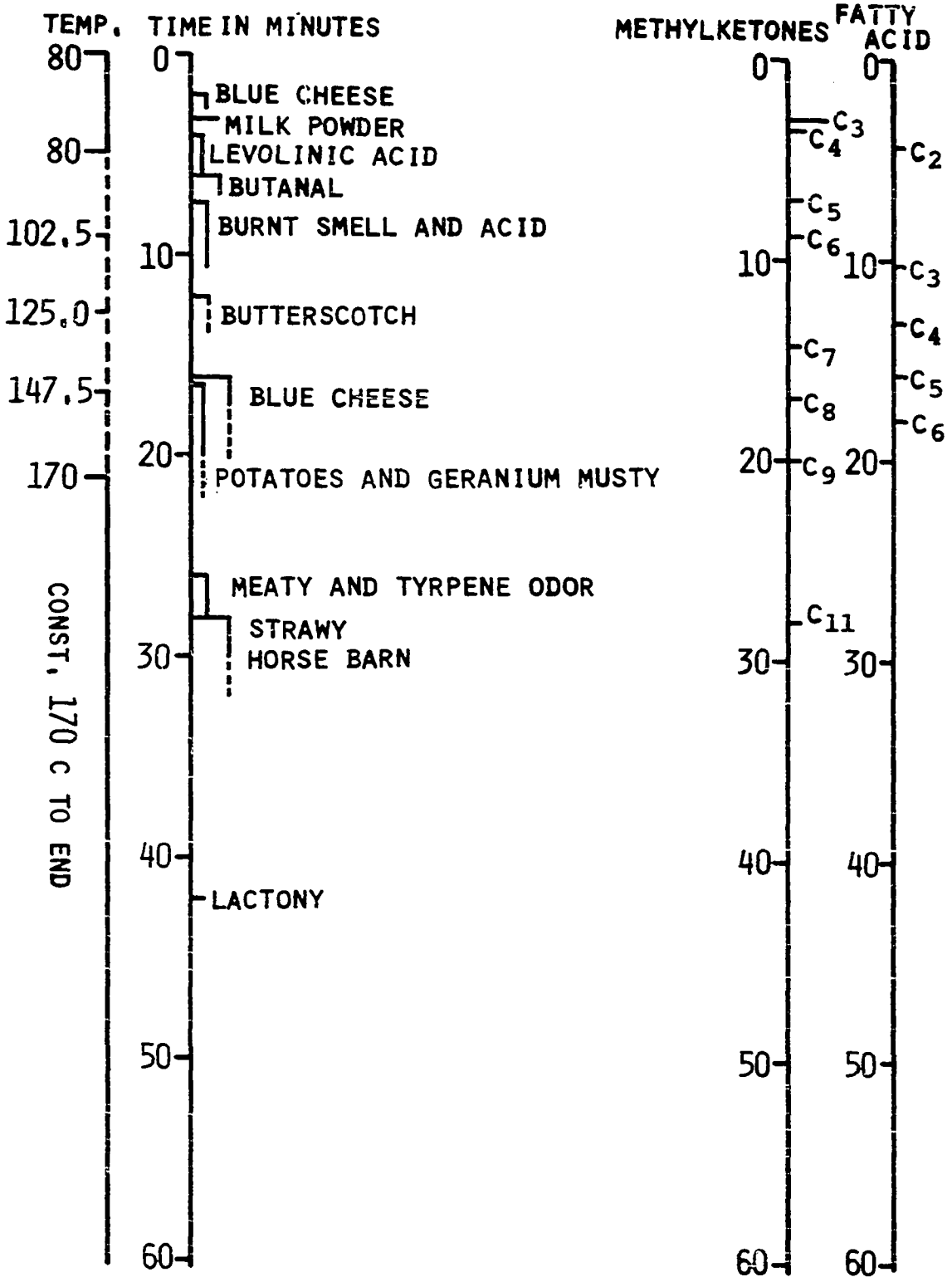

TEMP.
TIME IN MINUTES
METHYLKETONES
FATTY ACID
80
80
102.5
125.0
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
BLUE CHEESE
MILK POWDER
LEVOLINIC ACID
BUTANAL
BURNT SMELL AND ACID
BUTTERSCOTCH
BLUE CHEESE
POTATOES AND GERANIUM MUSTY
MEATY AND TYRPENE ODOR
STRAWY
HORSE BARN
LACTONY
C3
C4
C5
C6
C7
C8
C9
C11
C2
C3
C4
C5
C6

Figure 15. Organoleptic analysis of milk fat and deodorized milk fat cheese volatiles from experiment 3 separated on a 30% butanediol succinate column

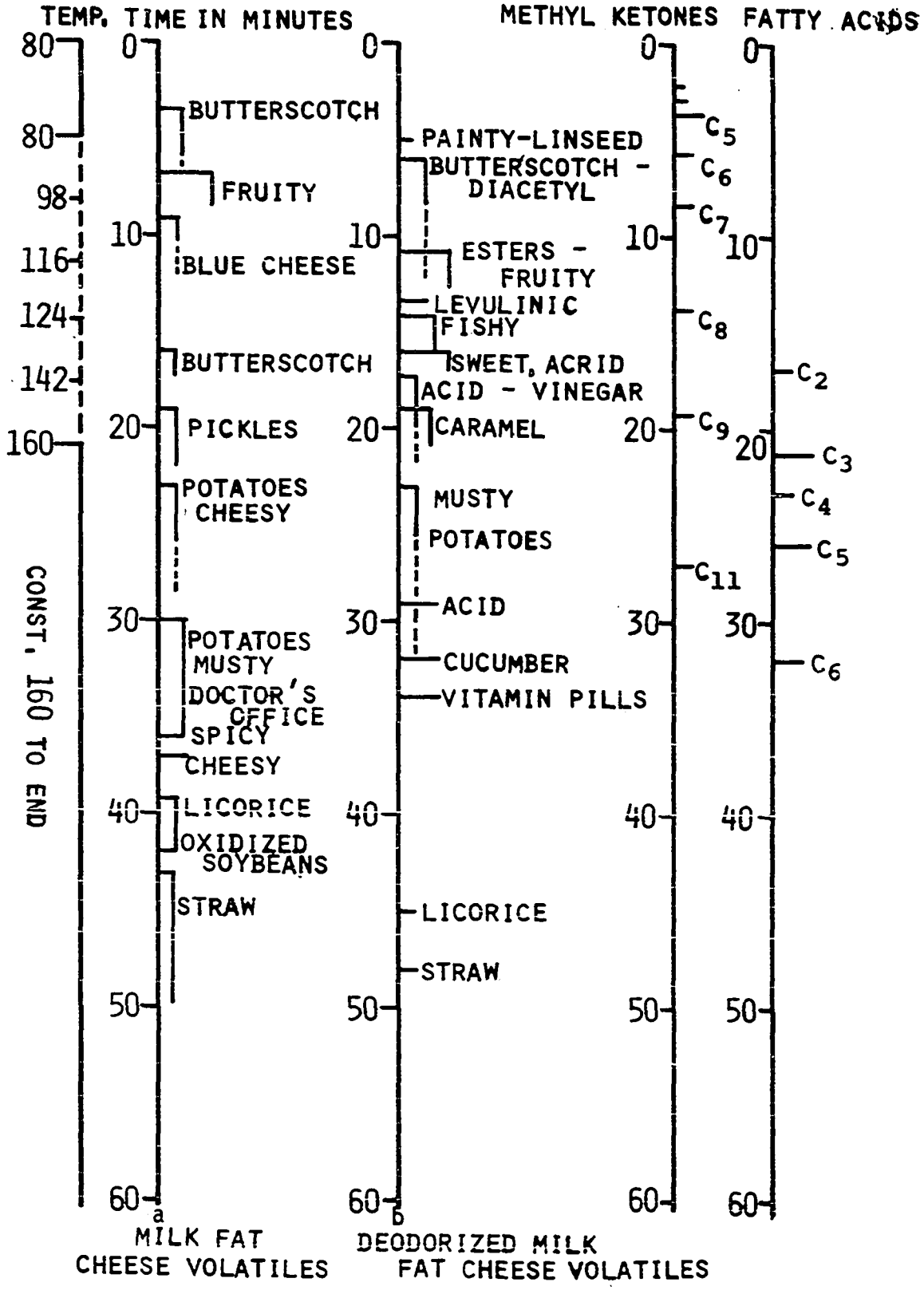

TEMP.
TIME IN MINUTES
METHYL KETONES
FATTY ACIDS
80
80
98
116
124
142
160
CONST. 160 TO END
0
10
20
30
40
50
60
BUTTERSCOTCH
FRUITY
BLUE CHEESE
BUTTERSCOTCH
PICKLES
POTATOES CHEESY
POTATOES MUSTY
DOCTOR'S OFFICE
SPICY
CHEESY
LICORICE
OXIDIZED SOYBEANS
STRAW
a
MILK FAT CHEESE VOLATILES
PAINTY-LINSEED
BUTTERSCOTCH - DIACETYL
ESTERS - FRUITY
LEVULINIC
FISHY
SWEET, ACRID
ACID - VINEGAR
CARAMEL
MUSTY POTATOES
ACID
CUCUMBER
VITAMIN PILLS
LICORICE
STRAW
b
DEODORIZED MILK FAT CHEESE VOLATILES
C5
C6
C7
C8
C9
C11
C2
C3
C4
C5
C6

Figure 16. Organoleptic analysis of conventional cheese volatiles separated on a 30% butanediol succinate column

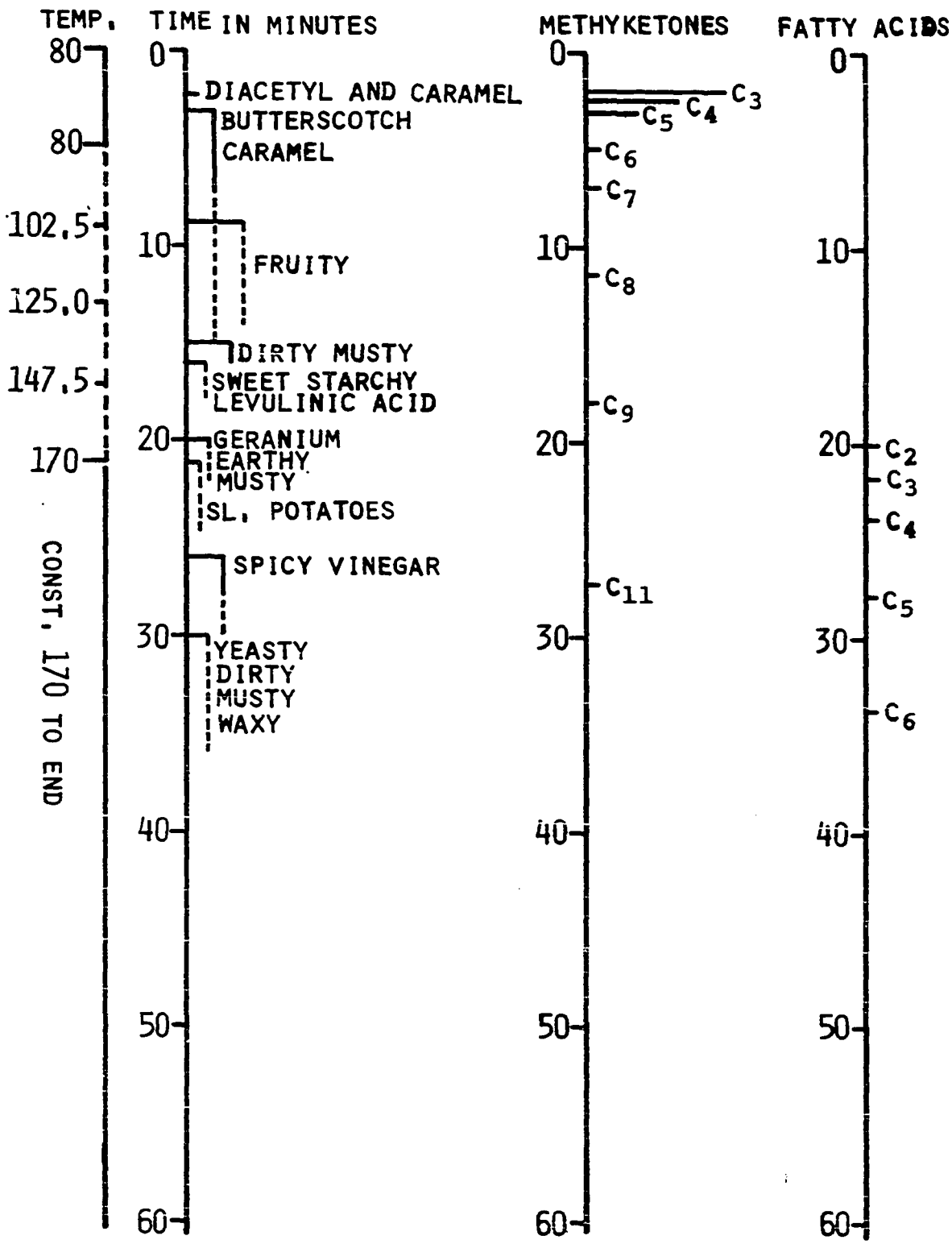

TEMP.
TIME IN MINUTES
METHYKETONES
FATTY ACIDS
80
80
102.5
125.0
147.5
170
CONST. 170 TO END
0
10
20
30
40
50
60
DIACETYL AND CARAMEL
BUTTERSCOTCH
CARAMEL
FRUITY
DIRTY MUSTY
SWEET STARCHY
LEVULINIC ACID
GERANIUM
EARTHY
MUSTY
SL. POTATOES
SPICY VINEGAR
YEASTY
DIRTY
MUSTY
WAXY
C3
C4
C5
C6
C7
C8
C9
C11
C2
C3
C4
C5
C6

Figure 17. Organoleptic analysis of conventional cheese one-day old separated on a 30% butanediol succinate column

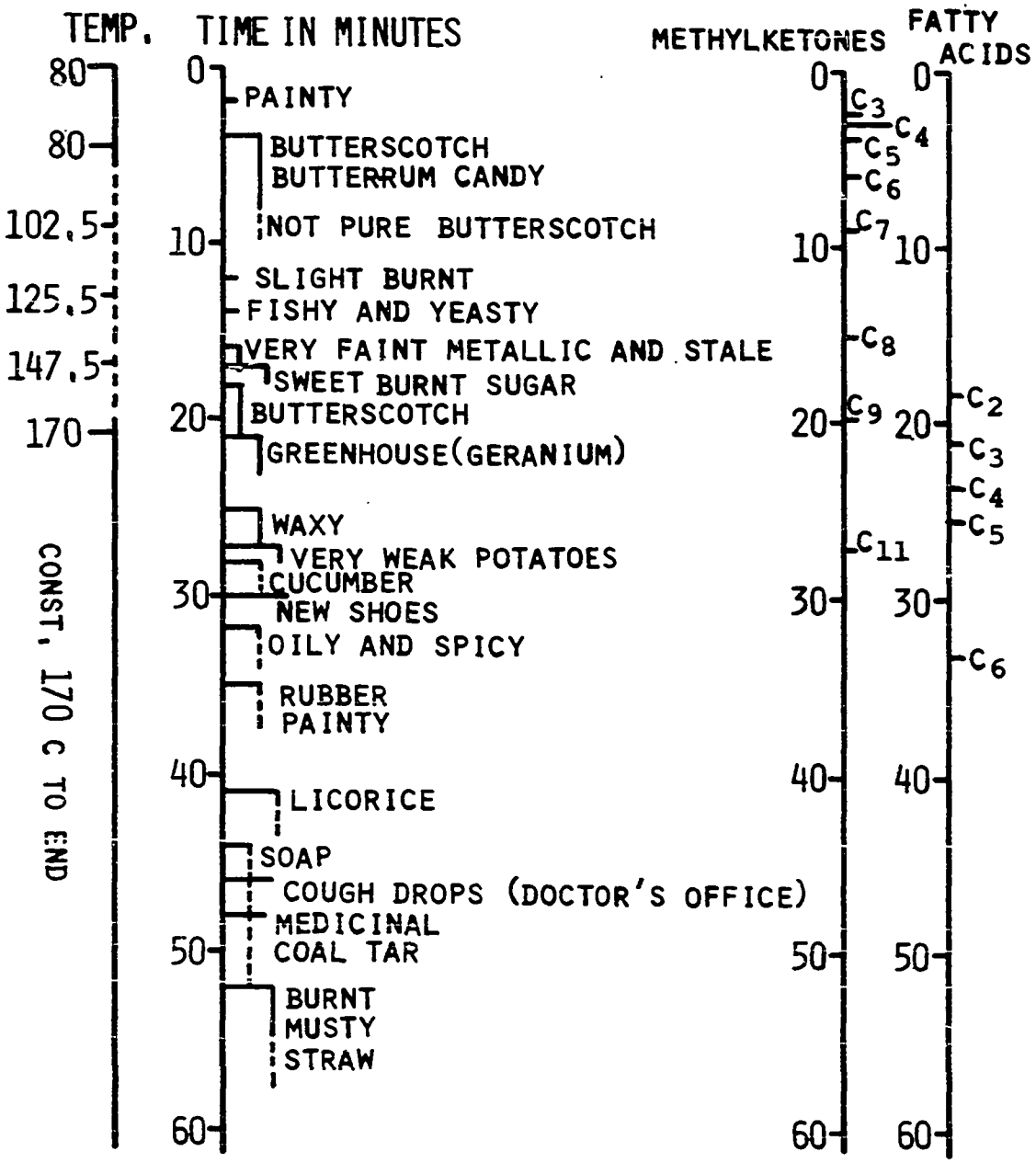

TEMP.
TIME IN MINUTES
METHYLKETONES
FATTY ACIDS
80
80
102.5
125.5
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
PAINTY
BUTTERSCOTCH
BUTTERRUM CANDY
NOT PURE BUTTERSCOTCH
SLIGHT BURNT
FISHY AND YEASTY
VERY FAINT METALLIC AND STALE
SWEET BURNT SUGAR
BUTTERSCOTCH
GREENHOUSE(GERANIUM)
WAXY
VERY WEAK POTATOES
CUCUMBER
NEW SHOES
OILY AND SPICY
RUBBER
PAINTY
LICORICE
SOAP
COUGH DROPS (DOCTOR'S OFFICE)
MEDICINAL
COAL TAR
BURNT
MUSTY
STRAW
C3
C4
C5
C6
C7
C8
C9
C11
C2
C3
C4
C5
C6

Figure 18. Organoleptic analysis of milk fat cheese volatiles from experiment 1 separated on a 30% butanediol succinate column

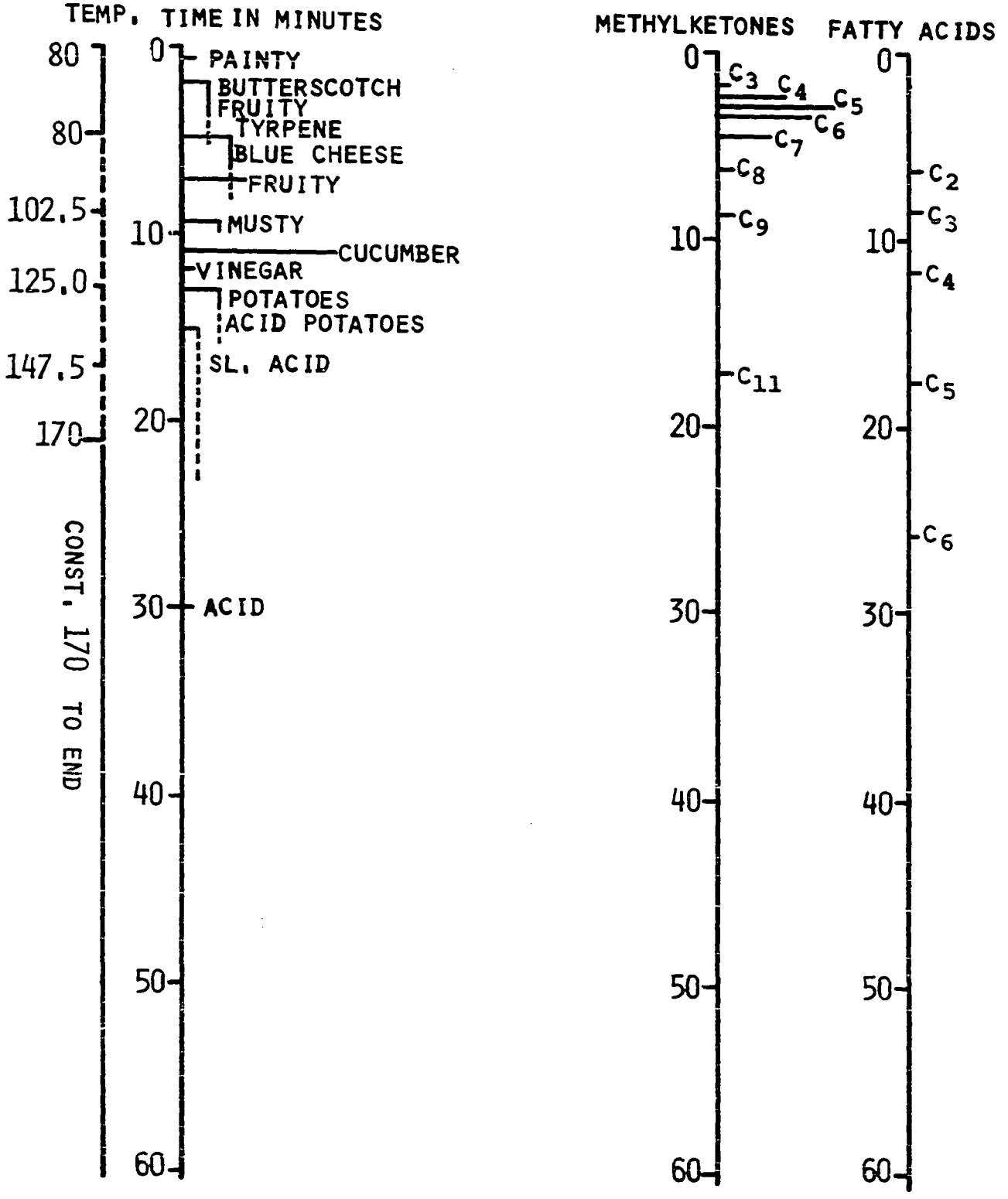

TEMP.
TIME IN MINUTES
80
80
102.5
125.0
147.5
170
CONST. 170 TO END
0
10
20
30
40
50
60
PAINTY
BUTTERSCOTCH
FRUITY
TYRPENE
BLUE CHEESE
FRUITY
MUSTY
CUCUMBER
VINEGAR
POTATOES
ACID POTATOES
SL. ACID
ACID
METHYLKETONES
FATTY ACIDS
C3
C4
C5
C6
C7
C8
C9
C11
C2
C3
C4
C5
C6

Figure 19. Organoleptic analysis of Kaola cheese volatiles from experiment 1 separated on a 30% butanediol succinate column

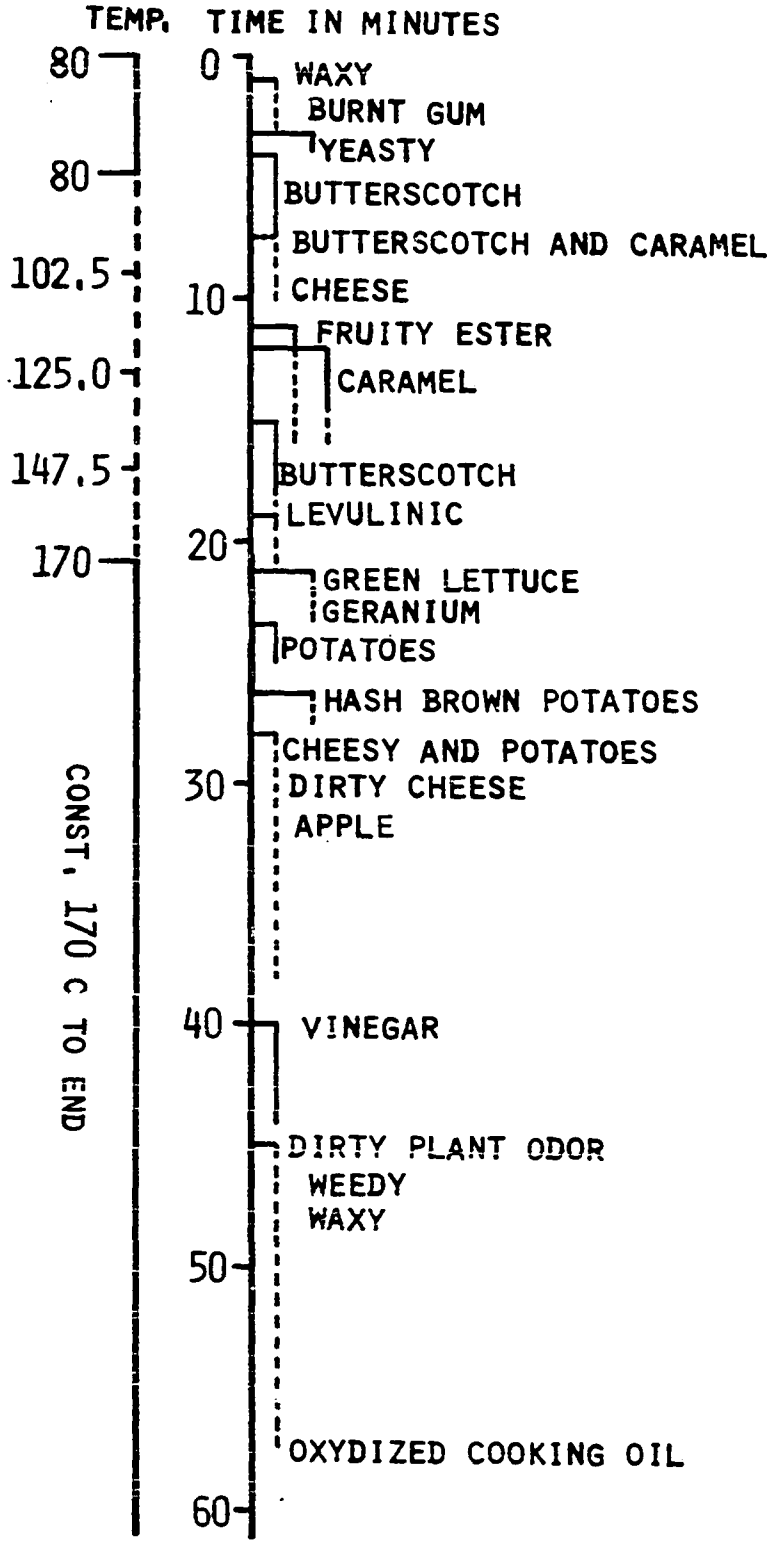

TEMP.
TIME IN MINUTES
80
80
102.5
125.0
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
WAXY
BURNT GUM
YEASTY
BUTTERSCOTCH
BUTTERSCOTCH AND CARAMEL
CHEESE
FRUITY ESTER
CARAMEL
BUTTERSCOTCH
LEVULINIC
GREEN LETTUCE
GERANIUM
POTATOES
HASH BROWN POTATOES
CHEESY AND POTATOES
DIRTY CHEESE
APPLE
VINEGAR
DIRTY PLANT ODOR
WEEDY
WAXY
OXYDIZED COOKING OIL

Figure 20. Organoleptic analysis of Kaomel cheese volatiles from experiment 1 separated on a 30% butanediol succinate column

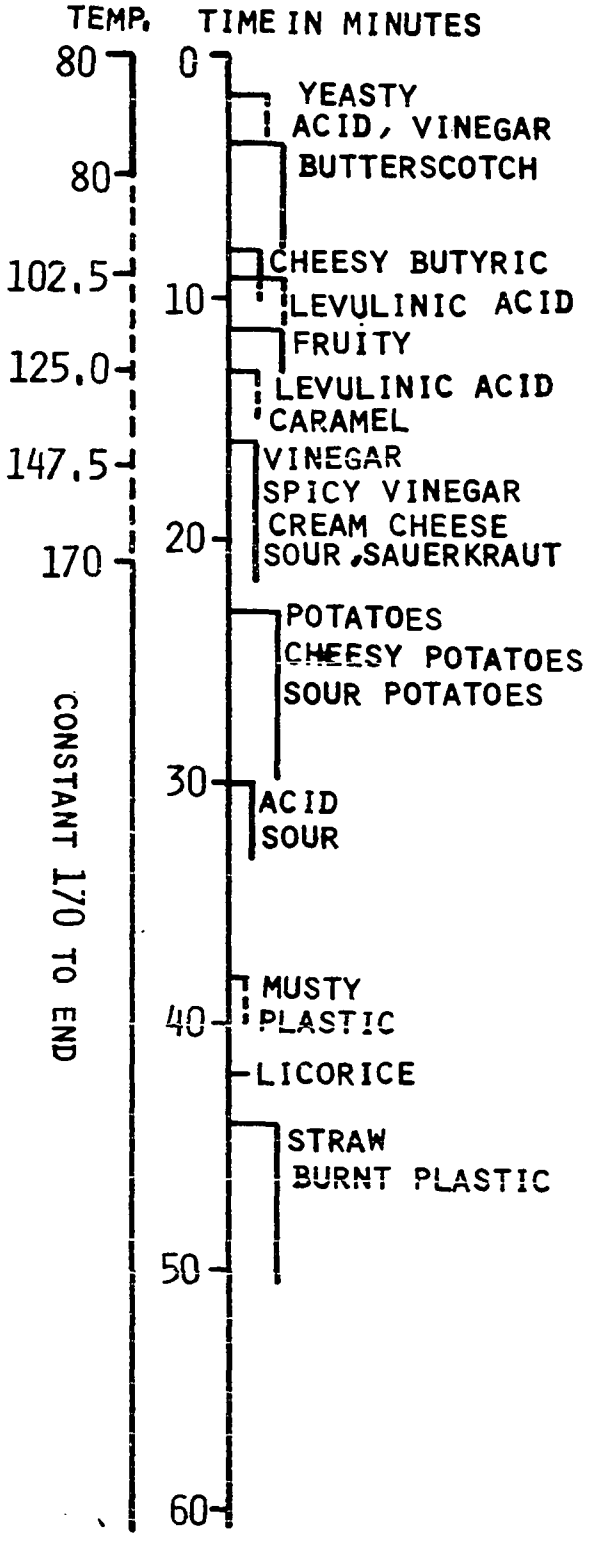

TEMP.
TIME IN MINUTES
80
80
102.5
125.0
147.5
170
CONSTANT 170 TO END
0
10
20
30
40
50
60
YEASTY
ACID, VINEGAR
BUTTERSCOTCH
CHEESY BUTYRIC
LEVULINIC ACID
FRUITY
LEVULINIC ACID
CARAMEL
VINEGAR
SPICY VINEGAR
CREAM CHEESE
SOUR, SAUERKRAUT
POTATOES
CHEESY POTATOES
SOUR POTATOES
ACID
SOUR
MUSTY
PLASTIC
LICORICE
STRAW
BURNT PLASTIC

Figure 21. Organoleptic analysis of mineral oil cheese volatiles from experiment 5 separated on a 30% butanediol succinate column

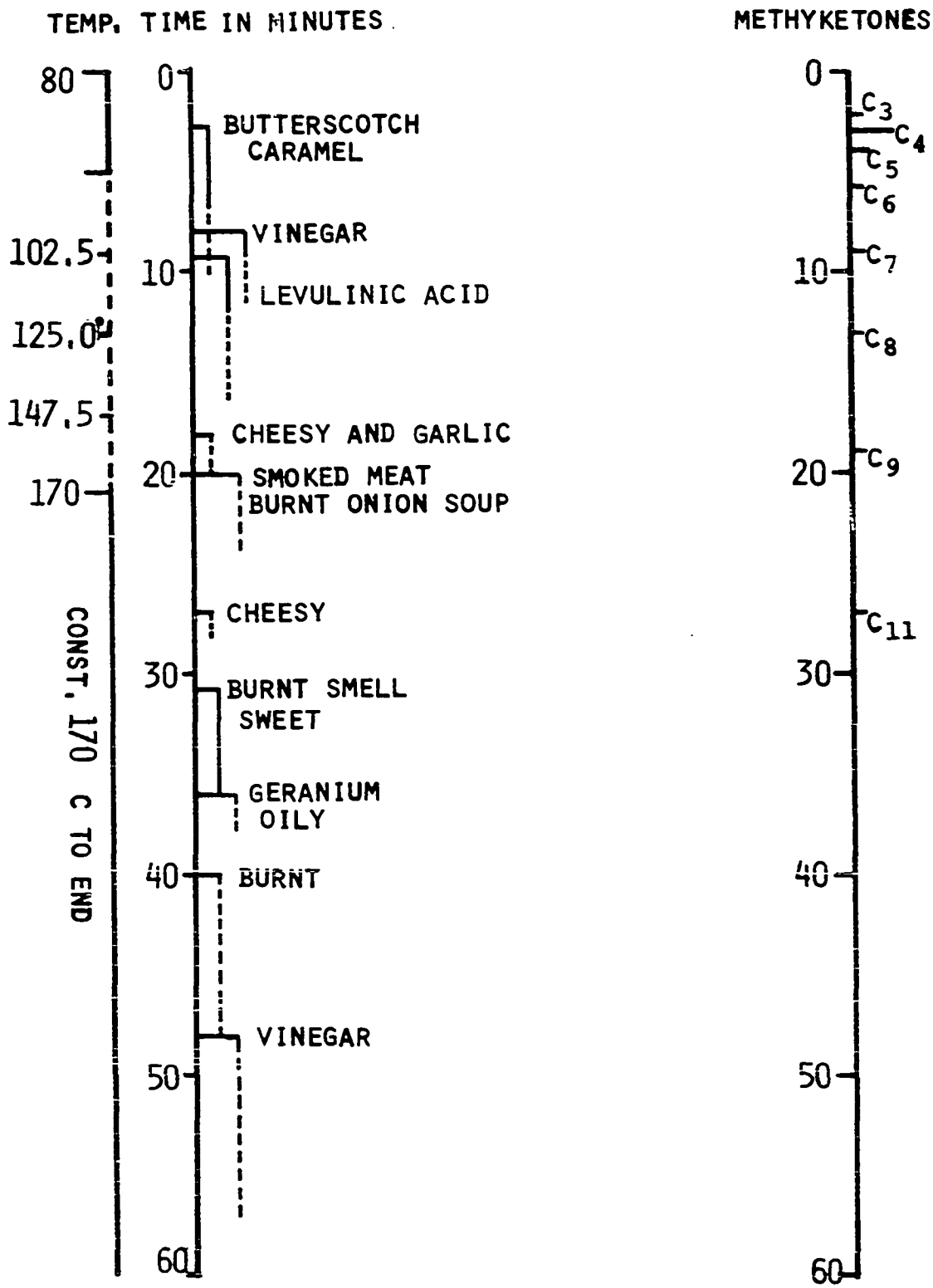

TEMP.
TIME IN MINUTES
METHYKETONES
80
102.5
125.0
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
BUTTERSCOTCH
CARAMEL
VINEGAR
LEVULINIC ACID
CHEESY AND GARLIC
SMOKED MEAT
BURNT ONION SOUP
CHEESY
BURNT SMELL
SWEET
GERANIUM
OILY
BURNT
VINEGAR
C3
C4
C5
C6
C7
C8
C9
C11

Figure 22. Organoleptic analysis of milk fat cheese volatiles from experiment 1 separated on a 30% butanediol succinate column

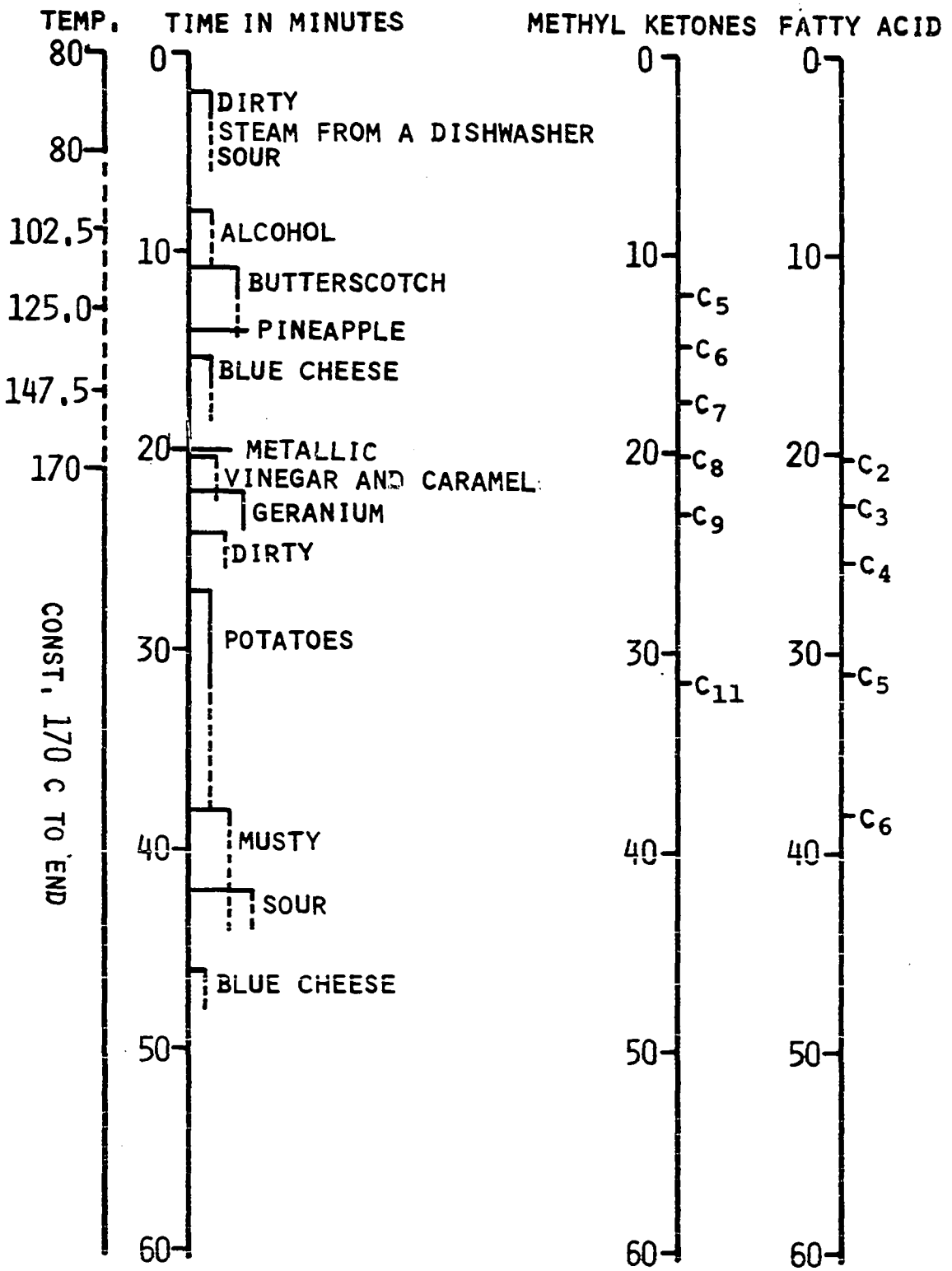

TEMP.
TIME IN MINUTES
METHYL KETONES
FATTY ACID
80
80
102.5
125.0
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
DIRTY
STEAM FROM A DISHWASHER
SOUR
ALCOHOL
BUTTERSCOTCH
PINEAPPLE
BLUE CHEESE
METALLIC
VINEGAR AND CARAMEL
GERANIUM
DIRTY
POTATOES
MUSTY
SOUR
BLUE CHEESE
C_5
C_6
C_7
C_8
C_9
C_{11}
C_2
C_3
C_4
C_5
C_6

Figure 23. Organoleptic analysis of milk fat cheese volatiles from experiment 1 separated on a 30% butanediol succinate column with a precolumn of 5% boric acid

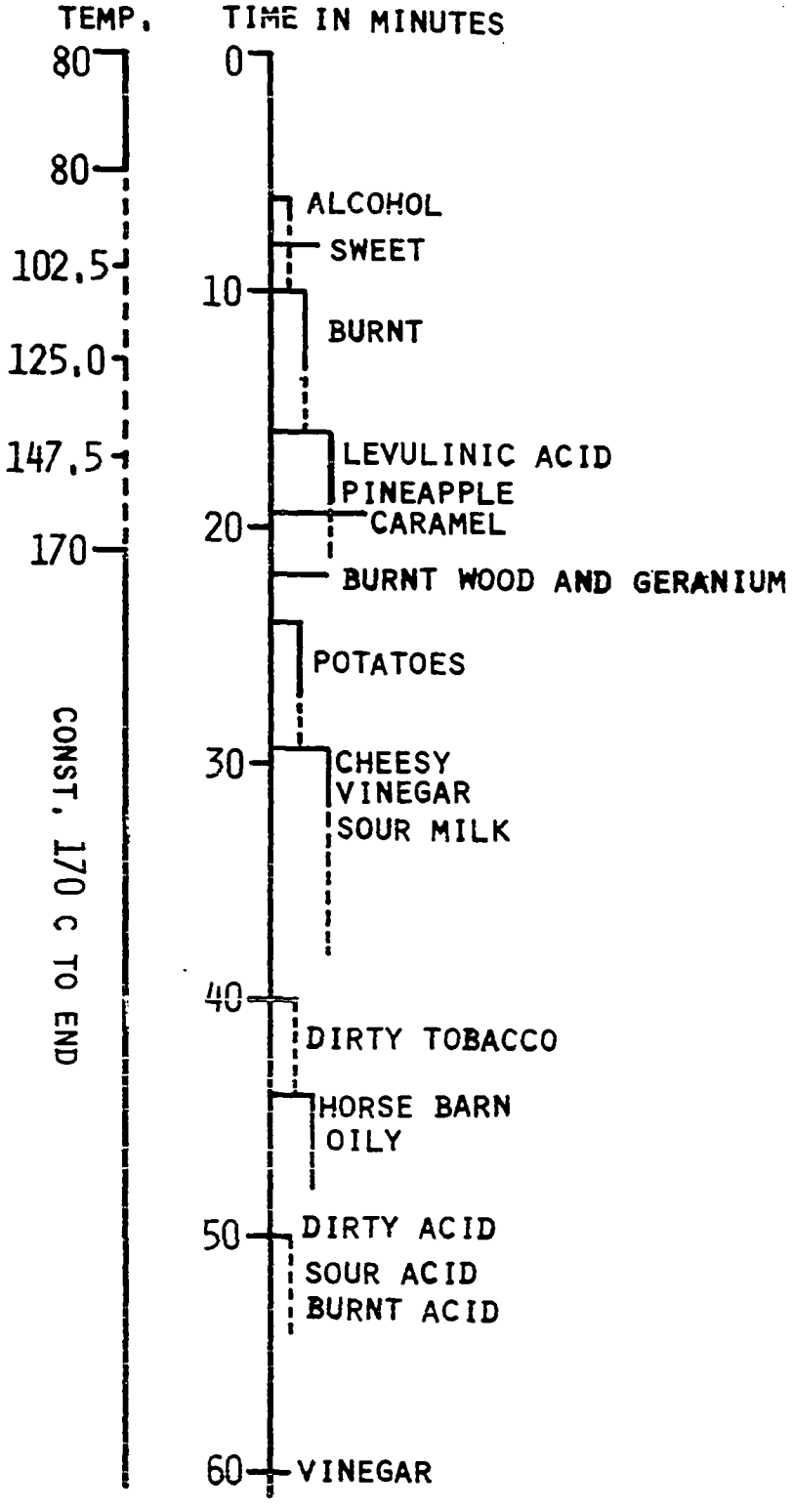
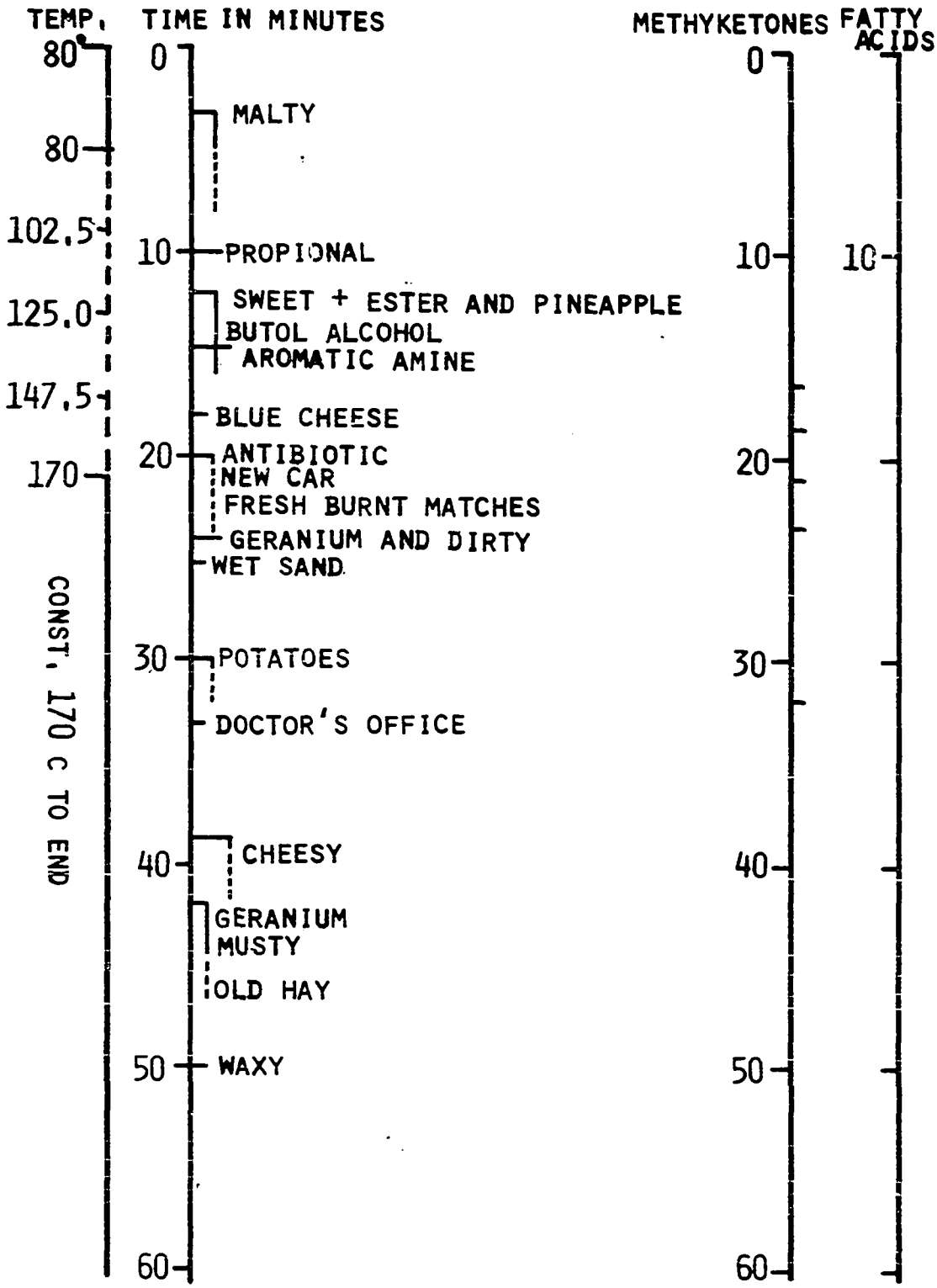

TEMP.
TIME IN MINUTES
80
80
102.5
125.0
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
ALCOHOL
SWEET
BURNT
LEVULINIC ACID
PINEAPPLE
CARAMEL
BURNT WOOD AND GERANIUM
POTATOES
CHEESY
VINEGAR
SOUR MILK
DIRTY TOBACCO
HORSE BARN
OILY
DIRTY ACID
SOUR ACID
BURNT ACID
VINEGAR

Figure 24. Organoleptic analysis of milk fat cheese volatiles from experiment 1 separated on a 30% butanediol succinate column with a precolumn of sodium borohydride


TEMP.
TIME IN MINUTES
METHYKETONES
FATTY ACIDS
80°
80
102.5
125.0
147.5
170
CONST. 170 C TO END
0
10
20
30
40
50
60
MALTY
PROPIONAL
SWEET + ESTER AND PINEAPPLE
BUTOL ALCOHOL
AROMATIC AMINE
BLUE CHEESE
ANTIBIOTIC
NEW CAR
FRESH BURNT MATCHES
GERANIUM AND DIRTY
WET SAND
POTATOES
DOCTOR'S OFFICE
CHEESY
GERANIUM
MUSTY
OLD HAY
WAXY

The most noticeable flavors of all the volatiles tested were two distinct butterscotch aromas, one with a retention time the same as methyl ketone of C_5 to C_7 and the other with a retention time the same as methyl ketone of C_8 to C_{10} on butanediol succinate. The second butterscotch aroma was usually combined with a caramel aroma. On apeizon L columns only one butterscotch component was noted. The Strecker degradation, a reaction between α-amino and glucose, develops a caramel aroma under heat (Rohan, 78; Schultz et al., 83).

Fruity aromas with a retention time similar to methyl ketone of C_6 to C_8 were frequently reported. Fruity flavor is probably an esters of fatty acids and ethanol. Suzuki et al. (91) suggested that esters were present, and recent work by Day and Libbey (19) confirmed their presence and importance in Cheddar cheese flavor. Ethyl butyrate and ethyl caproate were found to be the most important components. While these compounds occur in relatively high concentrations in normal flavored Cheddar cheese, fruity samples contain exceedingly high amounts (Schultz et al., 83).

A Blue cheese flavor with a retention time similar to methyl ketone of C_7 and C_8 was reported frequently. This is in agreement with Schultz et al. (83) that the methyl ketones, especially C_7 and C_9, are generally considered the key flavor of Blue cheese. Patton et al. (73) showed that the methyl ketone 2-heptanone is present in Cheddar cheese. On the other hand, Liebich et al. (54) found that the relative concentration of methyl ketones in Cheddar cheese is high, but not as predominant as in Roquefort cheese. This compound was usually reported only in milk fat or conventional cheese although it turned up in one Kaola sample. It should not

be found in deodorized milk fat and may account for the slight reduction in score of deodorized milk fat cheese.

An acetic acid, pickles, or vinegary aroma with a retention time similar to fatty acids of C_2 or C_3 (as shown in Figures 15 and 22) or similar to fatty acid of C_4 or C_5 (as shown in Figure 16) was reported. This indicates that these aromas are due to a mixture of volatile fatty acids, from caproic to capric, which contribute to the flavor of Cheddar cheese.

A cheesy aroma with a retention time similar to fatty acids of C_4 and up was sometimes reported. This aroma may be due to the volatile fatty acid or methional (3-methylthioproponal). In cheese methional might be generated by Strecker degradation using methionine. Methional has a cheesy-brothy flavor (Keeney and Day, 35) which could be very important in Cheddar cheese flavor.

Potatoes or musty aromas were usually reported with a retention time similar to methyl ketone of C_9 or more.

Figure 17 shows the organoleptic aroma analysis of a conventional Cheddar cheese one-day old on a 30% butanediol succinate column. This cheese gave more aroma than was expected. There were many off flavors such as metallic, greenhouse (geranium), wax, new shoes, etc. which were not noticeable with conventional cheese of 12-months old. These off flavors may be changed during ripening to a flavorful compound which could be responsible for Cheddar cheese flavor.

Figure 23 shows the organoleptic flavor analysis of milk fat on a 30% butanediol succinate column with a precolumn of 5% boric acid. The

boric acid precolumn should remove the alcohol as reported by Regnier and Huang (75). Strangely, this led to the report of an alcohol aroma which did not appear without using a precolumn of boric acid as shown in Figure 22.

A precolumn of sodium borohydride should subtract alcohols, aldehydes, and most of the ketones from the volatiles (Regnier and Huang, 75). Figure 24 shows the aroma obtained from milk fat cheese volatiles. The aroma of proponal was noticed, so some of the aldehyde was left. Blue cheese aroma, i.e., methyl ketone, was noticed which probably means that the precolumn of sodium borohydride was not very efficient in subtracting ketones. Sodium borohydride and 5% boric acid precolumns had a great effect on the butterscotch aroma. The caramel flavor disappeared completely by using sodium borohydride precolumn, and the 5% boric acid precolumn reduced the caramel flavor markedly.

SUMMARY AND CONCLUSIONS

The object of this investigation was to study the precursors of Cheddar cheese flavor. To do this fats of various kinds and mineral oil were homogenized into pasteurized skim milk, and the milk was made into Cheddar cheese. The cheeses were judged periodically up to 12 months of age. The judges were selected on the basis of a statistical method that measured their consistency in scoring the cheeses.

In an attempt to get more insight into the differences in the flavors of the various types of cheese, the cheese volatiles were studied by gas liquid chromatography. To do this the fat was separated by centrifugation at 30,000 g at 40 C, and the volatiles were molecular distilled from the fat. The collected volatiles were transferred to a special stainless steel trap to be injected into the gas liquid chromatograph. A splitter with a ratio of 1:1 was attached to the end of the column. Half of the gas was conducted to a hydrogen flame detector and half was vented for organoleptic examination. Precolumn reactors were also used that selectively removed certain chemical classes. The extent of proteolysis of the cheeses was also determined at the end of the ripening period.

The data suggested that cheese prepared from homogenized milk fat into skim milk does not have the degree of proteolysis or as good a flavor as conventional Cheddar cheese.

Statistical analysis showed the length of ripening and type of fat used for making cheese were significant at the 1% level. Milk fat and deodorized milk fat were superior to other fats, except milk fat plus

gum acacia, in flavor development but were not significantly different from each other. Deodorized milk fats should be efficient in lactones and ketones said to be important in the flavor of dairy products. Cheese made from milk fat and added buttermilk solids was significantly lower than milk fat cheese at the 1% level.

The addition of the deodorizer distillate, which should contain lactones and ketones often said to be important in the flavor of dairy products, to the deodorized milk fat lowered the scores markedly. The deodorized milk fat cheese containing distillate was significantly lower than milk fat cheese, deodorized milk fat cheese, or deodorized milk fat cheese containing dimethyl sulfide at the 1% level.

Kaola (a commercial product meant to imitate milk fat in its content of short chain fatty acids) cheese and Kaomel (a very bland commercial product meant to imitate cocoa butter) cheese were scored very low. The addition of butyric and caproic acid to Kaola to make it even more like milk fat in its fatty acid composition did improve the flavor significantly.

A bland mineral oil was used for making cheese. The mineral oil cheese was significantly better than Kaola and Kaomel cheeses. This indicates that Kaola and Kaomel probably contain off flavor precursors that reduce their scores. It may be that there was a beneficial effect from the butter-like composition of Kaola but that this was cancelled by its off flavor precursors. On the other hand, the superiority of milk fat and deodorized milk fat to these commercial products may be caused by some unknown constituent. The addition of dimethyl sulfide, a potent

flavor compound reported in milk which would be removed by deodorization, to mineral oil lowered the score; however, the difference was not statistically different. On the other hand, the addition of dimethyl sulfide to deodorized milk fat, in a concentration of 40 ppm, improved the flavor and the difference between deodorized milk fat cheese and deodorized milk fat cheese containing dimethyl sulfide was significant at the 1% level.

The addition of fat globule membrane material to milk fat did not improve the flavor significantly. The same results were obtained by adding fat globule membrane material to mineral oil. The addition of gum acacia to milk fat improved its flavor and the difference was highly significant.

The extent of proteolysis for the types of cheese used in experiment 1 to 6 was lower than for the conventional cheese. Although the F-test showed the difference between the ten types of cheese and the conventional cheese was not quite significant at the 5% level, a t-test showed a significant difference between the conventional cheese and cheese made from milk fat, Kaola, Kaomel, mineral oil, mineral oil containing dimethyl sulfide, or deodorized milk fat containing dimethyl sulfide. The relation of flavor score and proteolysis index was not statistically significant, which indicates that one cannot be reliably predicted from the other.

Gas chromatographic separation of the cheese volatiles gave a consistent aroma pattern but showed more variations within types than between types. The most frequently encountered aromas resembled butterscotch,

fruit(y), Blue cheese, acid, vinegar (or pickles), and potatoes. The use of a precolumn reactor reduced the aroma, especially butterscotch. A conventional cheese one-day old gave more aroma than was expected.

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APPENDIX

Figure 25. Score sheet used by panelists for flavor evaluation of cheese

NAME OF JUDGE _____

TESTING DATE _____ ORIGINAL DATE _____

CHEDDAR-LIKE FLAVOR

SAMPLE NO.	NO	SLIGHT	MODERATE	PRONOUNCED
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

NO MEANS NO RESEMBLANCE TO CHEDDAR FLAVOR
SLIGHT MEANS A DEFINITE RESEMBLANCE TO CHEDDAR CHEESE
MODERATE MEANS A GOOD RESEMBLANCE TO CHEDDAR FLAVOR
PRONOUNCED MEANS ALMOST IDENTICAL TO CHEDDAR FLAVOR

Table 20. Chemical analysis for milk and experimental Cheddar cheese

Type of fat	Milk		
	Percent fat	Percent moisture	Percent solids
Experiment 1			
None (skim)	0.03	91.26	8.74
Milk fat	3.70	87.87	12.13
Kaola	3.50	88.39	11.61
Kaomel	3.60	88.62	11.38
Experiment 2			
None (skim)	0.05	90.65	9.35
Milk fat	3.75	87.34	12.66
Kaola	3.80	87.41	12.59
Kaomel	3.80	87.33	12.67
Experiment 3			
None (skim)	0.05	90.47	9.53
Milk fat	3.80	87.11	12.89
Milk fat + buttermilk solids	3.85	86.20	13.80
Deodorized milk fat	3.80	87.19	12.81
Deodorized milk fat + distillate	3.80	87.15	12.85
Experiment 4			
None (skim)	0.01	91.50	8.50
Milk fat	3.50	87.51	12.49
Kaola	3.55	87.35	12.65
Esterified Kaola	3.60	87.23	12.77
Experiment 5			
None (skim)	0.02	91.04	8.96
Milk fat	3.75	87.65	12.35
Mineral oil	3.80	87.77	12.23
Mineral oil + dimethyl sulfide/g	3.65	87.72	12.28
Experiment 6			
None (skim)	0.04	90.66	9.34
Milk fat	3.70	87.16	12.84
Deodorized milk fat	3.60	87.24	12.76
Deodorized milk fat + dimethyl sulfide/g	3.75	87.08	12.92
Deodorized milk fat + distillate	3.65	87.27	12.73
Experiment 7			
None (skim)	0.75	90.42	9.58
Milk fat + heated fat globule membrane	4.00	87.46	12.54
Milk fat + fat globule membrane	3.90	89.54	12.46
Mineral oil + heated fat globule membrane	4.00	87.40	12.60
Mineral oil + fat globule membrane	4.00	87.61	12.39
Experiment 8			
None (skim)	0.05	90.78	9.22
Milk fat	3.20	87.74	12.26
Milk fat + gum acacia	3.21	87.87	12.13

[a]Fat percent on dry basis.

Cheddar cheese				
Percent fat	Percent moisture	Percent solids	Percent F.D.B.[a]	Percent salt
39.0	32.02	67.98	57.37	1.64
37.5	33.44	66.56	56.34	1.68
38.5	31.49	68.51	56.20	1.66
37.0	34.48	65.52	56.47	1.51
37.0	34.36	65.64	56.37	1.96
38.0	33.11	66.89	56.81	1.61
37.0	36.29	63.71	58.08	1.59
35.5	35.48	64.52	55.02	1.74
36.5	34.15	65.85	55.43	1.61
38.5	33.69	66.31	58.06	1.48
36.0	34.35	65.65	54.84	1.65
35.0	34.97	65.03	53.82	1.50
35.0	34.98	65.02	43.83	1.50
37.5	35.17	64.83	57.84	1.68
39.5	34.57	65.43	60.37	1.75
39.5	35.61	64.39	61.35	1.70
37.0	32.73	67.27	55.00	1.78
36.5	33.57	66.43	54.95	1.74
37.5	33.55	66.45	56.43	1.84
37.5	32.92	67.08	55.90	1.74
41.0	32.34	67.66	60.60	1.77
36.0	33.49	66.51	54.13	1.85
38.0	34.43	65.57	57.95	1.88
39.0	35.31	64.69	60.29	2.07
31.0	36.21	63.79	48.60	1.87
31.0	35.88	64.12	48.35	1.84

Table 21. The fatty acid profile of butter fat, Kaola, and Kaomel

Fatty acid		Milk fat (Percent)	Kaola (Percent)	Kaomel (Percent)
Butyric	4:0	3.0	--	--
Caproic	6:0	1.8	0.2	--
Caprylic	8:0	1.4	3.1	--
Capric	10:0	3.0	2.5	--
Lauric	12:0	3.4	18.3	0.5
Myristic	14:0	12.4	6.9	0.5
Pentadecanoic	15:0	2.1	--	--
Palmitic	16:0	33.3	11.2	1.5
Margaric	17:0	1.6	--	--
Stearic	18:0	11.3	8.4	9.8
Oleic	18:1	22.8	44.0	87.3
Linoleic	18:2	1.5	5.4	0.2
Iodine value		30	42	60
Melting point		95 F	90 F	98 F