rivatives: linalol, nerol, geraniol,  $\alpha$ -terpineol, and four oxides of linalol; moreover, two other substances are present: one is probably dimethyl-3,7-octatrien-1,5,7-ol-3. The total amount of these substances represents 1-3 mg/l. in Muscat juices.

Each of these compounds has different organoleptic characteristics and an aroma which is not identical with that of Muscat, that is found correctly in the mixture of all these different substances. The threshold of these different compounds is between 100 and >6000  $\mu$ g/l. Geraniol and linalol are, in the whole aroma, the most important substances, not only because they are the most concentrated. but also because they have the lowest thresholds. In the whole aroma, the different terpenes react with each other because the mixtures are more aromatic than each individual compound. The most aromatic of these substances can be involved in chemical transformations which lead to other terpenes less aromatic. These chemical transformations must result in the losses of aroma often observed during the processing or the storage of commercial juices and wines.

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### Cheddar Cheese Flavor. A Review of Current Progress

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Research on Cheddar cheese flavor from 1968 to 1974 is reviewed with respect to factors affecting flavor development, flavor volatiles identified, and the divergent views on the volatile components

During the period from 1968 to 1974, work on cheese flavor has been reviewed from several points of view. Schormuller's 1968 review deals with the chemistry and biochemistry of a variety of cheeses. Fryer (1969) published a comprehensive review of the microflora of Cheddar cheese and its influence on flavor. Forss (1969) reviewed the flavor of dairy products, including pertinent current scientific and patent literature on Cheddar flavor. Sandine and Elliker (1970) reviewed flavor in fermented dairy products, including Cheddar cheese. Reiter and Sharpe (1971) provided an update on the work being done at the National Institute for Research in Dairying in England, using aseptic cheese vats. Evans (1972) also reviewed the subject, but with greater emphasis on analytical aspects. Dwivedi (1973), in a review on the role of enzymes in flavor of dairy products, briefly deals with Cheddar cheese. There is also a review of cheese flavor in general, by Panouse et al. (1972), and an extensive review of flavor development in Swiss cheese by Langsrud and Reinbold (1973a-c, 1974).

In this review an attempt is made to summarize and critically assess recent work on factors involved in the development in Cheddar flavor and the flavor significance of components that have been identified.

most significant to the characteristic Cheddar aroma. Recent reexaminations of methods of isolating cheese volatiles are noted.

Early work on Cheddar flavor was based on the hypothesis that there was one compound or one class of compounds that provided the characteristic Cheddar flavor. Since such a compound could not be found, Kosikowski and Mocquot (1958) formalized the component balance theory, the essence of which had been stated earlier by Mulder (1952). This theory proposed that cheese flavor was produced by a blend of compounds, no one of which produced the characteristic flavor. If the proper balance of components was not achieved, then undesirable or defective flavors occurred. The component balance theory, to my knowledge, has not been questioned in recent years.

There are essentially two approaches to the study of Cheddar flavor. One approach is to isolate and identify components which contribute to the flavor. The other approach is to determine the factors or agents which influence or control the development of flavor.

#### FACTORS INFLUENCING DEVELOPMENT OF FLAVOR

The work being done at NIRD in England by Reiter and Sharpe (1971) is of particular interest. They make cheese in aseptic vats using  $\delta$ -gluconic acid lactone as the acid-producing agent. No starter culture is used, and the resulting cheese is completely devoid of Cheddar flavor. This of course demonstrates that they have eliminated the previously uncertain influences of chance contamination by bacteria from the cheese plant environment. It also demon-

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strates that enzymes which survive pasteurization of the milk and the rennet enzymes, by themselves, do not produce Cheddar flavor.

When starter is used in the aseptic vat in place of the gluconic acid lactone, at 6 months of age a mild but characteristic Cheddar flavor is produced, and at 12 months the flavor is fairly strong. So it is now clear that the starter organisms do contribute to the development of the flavor.

With no outside interference from contaminating organisms from the air and the plant equipment, we can be more confident in their demonstrations that different single-strain starter cultures produce different flavors. They also confirmed that organisms isolated from commercial cheese or milk, added along with the single-strain starter culture, increase the flavor intensity, and that contamination from the atmosphere can significantly increase the degree of flavor development.

Small aseptic vats, yielding 100 g of cheese, have been used to show that herd, season, feed, and method of milking influence the free fatty acid content of cheese. However, the small cheeses were kept at room temperature to accelerate ripening. The higher-than-normal ripening temperature might produce changes in fatty acid specificity and pH optima of lipases. So the results from cheese ripened at normal temperatures might have been different, but Reiter and Sharpe's conclusion that the composition of the milk influences the FFA content of cheese is probably still valid.

Reiter and Sharpe have also obtained data that both support and disagree with results reported by Ohren and Tuckey. Ohren and Tuckey (1969) concluded that fat is essential to the development of flavor, and that more free fatty acids are produced in cheese when the milk, prior to pasteurization, has a higher microbial population. They also suggest that the total FFA and/or the ratio of acetate to total FFA must be within certain limits to obtain desirable Cheddar flavor. Reiter and Sharpe confirmed that higher bacterial populations in the milk prior to pasteurization increase the flavor development as well as the FFA content of the cheese. But they could not agree with the correlation of the restricted FFA range with the quality of the cheese flavor.

Two other groups have studied the role of fat in cheese ripening. Foda et al. (1971, 1974) substituted synthetic fats (Kaomel and Kaola) and mineral oil for milk fat. They concluded that the major role of the fat is to dissolve and hold the flavor components, although the state of emulsification of the fat affected flavor development. When milk fat was reincorporated into skim milk, the flavor was improved by using gum acacia as an emulsifying agent. They suggest that the fat-water interface has an important influence on flavor development.

Deane (1972) and Deane and Dolan (1973) also found that flavor does not develop in low fat cheese. They report decreased production of carbonyls in low fat cheese, principally in the methyl ketones but also in the aldehydes.

Another very active group is that of Kristoffersen and coworkers (Kristoffersen, 1973). Their hypothesis is that the state of oxidation of the protein sulfur in the milk is a major determinant in the development of cheese flavor. They suggest that the oxidized or disulfide form of sulfur acts as an acceptor of hydrogen, thereby promoting oxidative processes involved in the production of essential flavor components. They find that active sulfhydryl groups increase in proportion to the degree of flavor development. The flavor development may not always be desirable, but in cheese with acceptable flavor quality, the ratio of hydrogen sulfide to free fatty acids falls within certain limits.

In experiments with slurries of fresh cheese curd or of acid precipitated curd inoculated with a lactic culture, cheese flavor could be produced in 4 to 7 days. Flavor production was increased by adding reduced glutathione, cobalt, and a mixture of diacetyl or citrate, plus manganese and riboflavine. Other factors found to be important to flavor development were the type of acidulant, pH control, and the presence of active rennet. With respect to the production of volatile flavor compounds, the observed effects might not apply to normal cheese since the slurry constitutes quite a different system. Air is periodically stirred in, the water content is higher (55–60% vs. normal 34–36%), and the slurry is maintained at 30°. Nevertheless, cheese flavor is produced, and at an accelerated rate. Further studies of slurries may be very productive in establishing the mechanisms of flavor development.

Accelerated ripening of Cheddar has also been achieved by Iwasaki and Kosikowski (1973) by addition of mixtures of commercial microbial enzymes to pasteurized cheese curd.

# IDENTIFICATION OF COMPOUNDS ESSENTIAL TO CHEDDAR FLAVOR

There is some agreement that there are certain key compounds that have not been identified or whose importance has not been clearly established or sufficiently emphasized. However, there seems to be some difference of opinion as to where those key compounds are likely to be found.

Manning and Robinson (1973) claim that the characteristic flavor can be produced by the low molecular weight fraction of Cheddar volatiles. Wong et al. (1973) and O'-Keefe and coworkers (O'Keefe et al., 1969; O'Keefe, 1972) have been interested in the high boiling point fraction. Liebich et al. (1970) suggest that free fatty acids are the basis for any cheese flavor, and that the characteristic aroma is due to the ratio of FFA and, in particular, to the composition of the mixture of other volatile components. From our own published work (McGugan et al., 1968) we would appear to agree with Manning and Robinson, insofar as we found only two low boiling point sulfides to relate to the presence of Cheddar flavor. But the intact aroma of the cheese distillates was no longer present when the total effluent was recovered from our GC columns.

Manning and Robinson vacuum distilled fat from 7–10month-old cheese described as mild mature Cheddar. The vapors that passed through a  $-80^{\circ}$  trap were collected in a liquid nitrogen trap. When the liquid nitrogen trap was allowed to warm up, the vapors emerging between -100 and  $-70^{\circ}$  smelled strongly of cheese. They comment that the opinion that the odor could be described as Cheddar was not unanimous. Through noting odors from the gas chromatographic effluent and use of retention times and mass spectral data, they identified H<sub>2</sub>S, methanethiol, dimethyl sulfide, and diacetyl in the fraction with the cheeselike odor. They make the point that fatty acids are not necessary to produce the cheese aroma, but they concede that fatty acids, particularly butyric, may contribute to the total flavor.

In a subsequent paper, Manning (1974) deals with the three sulfur compounds. Methanethiol was present in two cheeses made with starter that had normal Cheddar flavors, but was absent in the gluconic acid lactone cheese and skim milk cheese, both of which were totally lacking in Cheddar flavor.

Hydrogen sulfide was present in all four cheeses, but was at about twice the level in the cheese with normal flavors. Dimethyl sulfide was also present in all cheeses, but in about equal concentrations in one starter cheese and the gluconic acid lactone cheese. Manning concluded that methanethiol was the most significant component, and that  $H_2S$  and dimethyl sulfide might contribute to the full Cheddar flavor but they did not seem to be essential to the production of the cheesey aroma.

O'Keefe et al. (1969) isolated a "coconut" aroma fraction from the high boiling point neutral volatiles. In it they identified a series of  $\delta$ - and  $\gamma$ -lactones from C<sub>10</sub> to C<sub>18</sub> (nine in all). The lactones constituted 70% of the high boiling point compounds in a molecular distillate of cheese fat. They tested the influence of the lactones by adding them to a simulated cheese made by blending carbonyls, acids, and 3-mercaptopropionic acid with safflower oil and cottage cheese curd. The Cheddar aroma of the simulated cheese was enhanced by  $\delta$ -dodecalactone and  $\delta$ -tetradecalactone at 4 and 1.5 ppm, respectively. However, they made cheese, some with added lactobacilli, and some without. The lactobacilli cheese developed a more pronounced Cheddar flavor, but the lactobacilli had little influence on the production of neutral high boiling point compounds.

Wong et al. (1973) found a correlation between the number and quantity of lactones and the age and flavor of cheese.

Liebich et al. (1970) identified about 120 compounds in Cheddar volatiles. They comment on the relatively high concentration of terpenes. Their list also contains four  $\delta$ lactones. No  $\gamma$ -lactones were found and only one sulfur compound (ethyl mercaptan) was tentatively identified.

Keen and Walker (1974) studied diacetyl, acetoin, 2,3butylene glycol, butanone, and 2-butanol in ripening cheese. They concluded that diacetyl was present in cheese up to the age of 1 year at levels that would have an influence on the flavor (over 5 months age, 0.5-1.5 ppm). Butanone concentrations varied widely in good flavored cheese, so it was not considered to be a likely contributor to the flavor.

In another paper devoted to methyl ketones (Walker and Keen, 1974), they concluded that methyl ketones may contribute to the flavor but, by themselves, they do not impart a typical Cheddar flavor to cheese.

Gray and Walker (1972) also concluded that  $C_2-C_{10}$  fatty acids do not play an important part in the typical flavor of mature Cheddar.

Bradley and Stine (1968) detected nine sulfides in natural Cheddar by subjecting distillates to gas chromatography before and after removing sulfides from the distillates by treating them with mercuric chloride. Only seven sulfur compounds have been identified in Cheddar volatiles [H<sub>2</sub>S, CH<sub>3</sub>SH, CH<sub>3</sub>SCH<sub>3</sub>, CH<sub>3</sub>SSCH<sub>3</sub>, CH<sub>3</sub>SCH<sub>2</sub>CH<sub>2</sub>CHO, CH<sub>3</sub>CH<sub>2</sub>SH—tentative (Liebich et al., 1970), and 2,3,4-trithiapentane—tentative (McGugan and Howsam, unpublished)], so there may be a few unidentified sulfides.

Tanaka and Obata (1969) cultured Aspergillus flavus with individual amino acids. They found that L-leucine and L-methionine produced  $\alpha$ -ketoisocaproic acid and  $\alpha$ -keto- $\beta$ -methylmercaptobutyric acid, respectively. A mixture of these two compounds has a cheeselike flavor.

Badings et al. (1968) found a phenolic flavor defect in Gouda cheese to be due to p-cresol produced by salt tolerant lactobacilli in the rennet.

Monais et al. (1973) and Adda and Dumont (1974) also reported phenolic compounds in cheese: phenol in Pont l'Eveque, phenol and cresol in Livarot, and phenol, cresol, and *p*-ethylphenol in Camembert.

Our own analyses have shown phenol (unpublished) and cresol (McGugan and Howsam, 1973) to be present in old Cheddar.

It is also interesting to note that phenolic compounds are included in a patented formulation for Cheddar flavor. Phenol, cresol, and O-methoxyphenol (guaiacol) are emphasized, but 12 others may be used (Pintauro, 1971).

Pyrazines are another class of compounds that may contribute to cheese flavor. On the basis of GC retention times and the distinctive odors in the GC effluent, we are fairly confident that 2-acetylpyrazine and 2-methoxy-3-ethylpyrazine are present in distillates from old Cheddar. There are several others present that have nutty odors but the odors are not distinctive enough to allow us to name them. All of the pyrazines are present at low levels, but collectively they may have a beneficial effect on Cheddar flavor. However, we have also noted pyrazine-like odors in the volatiles from a sterilized skim milk culture medium, so at least some of them may be present in the milk used for cheesemaking.

In 1957, Keeney and Day distilled protein hydrolysates in the presence of pyruvic acid, insatin, or ninhydrin. During the course of their experiments, the air became permeated with distillate odors which, with time, diminished to a pronounced odor of toasted cheese. They attributed the cheesey odor to methional and other aldehydes that might have been formed by the Strecker degradation. In view of present knowledge of the production of pyrazines from amino acids and the fact that some pyrazines have extremely low odor thresholds, there is a possibility that pyrazines had played some part in the production of the cheeselike odor.

On the subject of isolating and analyzing volatiles from cheese, only a few general references will be cited and a few variations of methods applied to cheese will be commented on.

Although not dealing specifically with cheese, there is an excellent discussion of methods by Weurman (1969), and the book of flavor research by Teranishi et al. (1971) can be recommended. Two techniques are suggested as ones which might be used to advantage more often, i.e. freeze concentration and absorption on charcoal.

Chang (1973) has summarized the flavor research techniques used at Rutgers University, and a symposium on nonvapor techniques includes papers on laser Raman spectroscopy (Freeman, 1973), high pressure liquid chromatography (Molyneux and Wong, 1973), and gel permeation (Schmit et al., 1973). Some of these techniques may prove useful in Cheddar flavor work.

There have been a few recent reexaminations of methods for the isolation of cheese volatiles. Dumont and Adda (1972) and Adda and Dumont (1972) studied high vacuum distillation of cheese fat, and a reflux distillation of wholecheese slurries. They concluded that methods based on separation of the fat result in serious losses of low molecular weight and water-soluble compounds. No one method was considered satisfactory for the full range of volatiles.

Manning and Robinson (1973) have adopted vacuum distillation of frozen grated whole cheese. They have focused their interest on the fraction that is volatile at  $-80^{\circ}$ .

Others are analyzing for specific classes of compounds methyl ketones, lactones, etc., as mentioned previously.

In our own work, we have stayed with analysis of volatiles vacuum distilled from the cheese fat. We recognize that we are discriminating against the most highly volatile components and against the water-soluble components. But in terms of the flavor of mature raw-milk Cheddar, we feel that most of the important low molecular weight compounds have been identified, and that there are a number of unidentified components in the medium boiling point range that may be significant. When we smell the GC effluent, we noted a number of odors which we could not account for on the basis of compounds known to be in Cheddar. From the nature and strength of the odors and from the fact that we cannot find a combination of known components that will reproduce the Cheddar aroma, we feel that some of these unidentified components may be important.

There are obviously very divergent views on methods of attacking the problem, and there are divergent views on the components likely to have the major influence on Cheddar flavor. This might stem from the fact that different groups are working on Cheddar cheese with different degrees of flavor development, and with quite different flavor characteristics. I suspect that the compounds required to produce the flavor of Cheddar made from pasteurized milk in an aseptic vat, with a single-strain starter culture, would not, in any combination of concentrations, produce the flavor of a mature raw-milk commercial Cheddar cheese. The group at NIRD may be quite right when they downgrade the importance of free fatty acids in their aseptic cheese. It is probably true also that, in pasteurized milk cheese typical of the United States market, H<sub>2</sub>S and fatty acids are important contributors to the flavor. In old raw-milk Cheddar other components such as phenolics and, perhaps, pyrazines also may be significant.

When we have identified all of the volatile components that occur in significant quantities in Cheddar, we may be able to determine the combinations that are essential to reproduce the various qualities and intensities of flavor that occur. At that time, those working on mechanisms of flavor development will no longer have to be guided by correlations that may often have little to do with the production of essential flavor components.

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Other papers presented at the 168th National Meeting of the American Chemical Society in the Symposium on Flavor Chemistry of Processed Foods but not printed in this issue are: "Flavor Analysis of Formulated Potato Chips", by Michael R. Sevenants and Charles C. Krause; "Higher-Boiling Volatiles in Canned Whole Kernel Sweet Corn", by L. M. Libbey, M. E. Morgan, L. A. Hansen, and R. A. Scanlan; and "The Flavor of Canned Meat. Influence of Processing Conditions and Storage", by Tyko Persson and Erik von Sydow.

## Forerunners of Pesticides in Classical Greece and Rome

Allan E. Smith\* and Diane M. Secoy

Various methods for pest control described by the classical writers are discussed. These include religion, folk magic, and the use of what may be termed chemical methods for the control of plant diseases, weeds, and insect and animal pests. These last are described in some detail and attempts are made to assess their possible success. Although the efficacy of such methods may be open to conjecture, the principles of seed treatment, fumigation, tree banding, and the use of preparations to control pests appear to have been widely used.

Although the science of pest control is considered to be of recent origin, dating from the latter part of the nineteenth century, it is probably true that man has practiced some form of pest control since the beginnings of agricultural times. The earliest implement, other than the hand, used for weed control may have been a stick for grubbing out unwanted plants in crops, while some form of whisk may have been used to remove troublesome insects.

During the classical ancient Mediterranean period there were several men whose writings on agricultural subjects have survived to modern times. Of these the Greeks Demo-

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