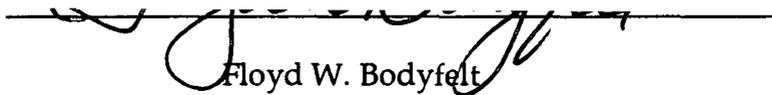


AN ABSTRACT OF THE THESIS OF

Scott A. Rankin for the degree of Doctor of Philosophy in Food Science and Technology presented on December 15, 1995. Title: Solvent Desorption Dynamic Headspace Analysis of Dairy Product Aroma Compounds.

Abstract approved:

  
Floyd W. Bodyfelt

A method for the assessment of volatile compounds in dairy products was developed using solvent desorption dynamic headspace sampling. The method was first applied to assay for diacetyl and acetoin in buttermilk. Major buttermilk volatiles recovered included diacetyl, acetic acid, and acetoin. Normalized detector responses were linear over the range of concentrations tested for diacetyl and acetoin. The method enabled quantitative estimation of diacetyl and acetoin in <30 min, including sample preparation time.

Next, the ability of stabilizing and emulsifying agents to inhibit the release of diacetyl from a model dairy matrix was examined using modified purge parameters. Stabilizers (guar, xanthan, and carrageenan) and emulsifiers (lecithin, carboxymethyl cellulose, and Tween 80) were examined for their effects on headspace available diacetyl at 0.05, 0.10, and

0.20% (wt/wt) in a 5% milkfat model system. Guar gum and carrageenan exhibited similar diacetyl release inhibition when corrected for viscosity. Xanthan gum exhibited the greatest decrease in headspace available diacetyl after correction for viscosity at increasing gum levels. Tween 80 imparted no significant viscosity and had no effect on recoverable diacetyl. Lecithin had no effect on viscosity, however it did inhibit the release of diacetyl as a function of lecithin level. Carboxymethyl cellulose increased viscosity and inhibited diacetyl release.

Finally, a rapid dynamic headspace sampling technique was evaluated for its ability to differentiate between Cheddar cheese samples for volatile aroma compounds. Seven samples of Cheddar cheese were examined ranging in flavor from mild to extra sharp. A total of 14 volatile compounds were tentatively identified with published retention indices and retention times of known standards. Major volatiles recovered were 2-butanol, acetoin, propanoic acid, butyric acid, and caproic acid. Other identified compounds were 2-butanone, diacetyl, ethyl butyrate, 1-butanol, ethyl caproate, hexanol, acetic acid, 2,3-butanediol, and octanoic acid.

The application of solvent desorption dynamic headspace sampling of dairy volatiles is a simple, rapid method for the determination of volatile compounds previously shown to influence flavor and aroma of dairy products. This research was conducted to demonstrate the optimized application of this technology to tracking dairy products aroma compounds.

Solvent Desorption Dynamic Headspace Analysis  
of Dairy Product Aroma Compounds

by

Scott A. Rankin

A DISSERTATION

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the requirements for the  
degree of

Doctor of Philosophy

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Doctor of Philosophy thesis of Scott A. Rankin presented on December 15, 1995

APPROVED:

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Major Professor, representing Food Science and Technology

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Head of Department of Food Science and Technology

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Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Scott A. Rankin, Author

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# SOLVENT DESORPTION DYNAMIC HEADSPACE ANALYSIS OF DAIRY PRODUCT AROMA COMPOUNDS

## CHAPTER 1. INTRODUCTION

Fermented dairy products rely on numerous volatile compounds to elicit desirable aroma. Some fermented dairy products are reported to rely on a small number of volatile compounds while other fermented dairy foods may rely on dozens or even hundreds of aroma-imparting compounds. In fact, over 200 volatile compounds have been identified in Cheddar cheese. These compounds result from the action of added (starter) and adventitious bacteria, endogenous and added enzymes, and from spontaneous chemical breakdown.

The presence or absence of these compounds at the appropriate levels is critical to obtaining a characteristic aroma. Hence, much research has been conducted to: (1) objectively determine the type and concentrations of these compounds, and (2) develop rapid methods that allow the routine analysis of dairy products for these compounds. Although much success has been gained in determining what types and levels of compounds are responsible for typical aroma, few methods exist for the routine analysis of fermented dairy product aroma compounds.

Solvent desorption dynamic headspace analysis is a technique developed and used for the assessment of volatile compounds of environmental interest or of toxicological concern. Since routine analysis of these volatile compounds is critical for tracking and assaying for these

important compounds, technologies and resources are in place for the routine analysis of these compounds.

The purpose of this research was to apply the technique of a solvent desorption dynamic headspace sampling system for the assessment of relevant volatile compounds found in dairy products.

## CHAPTER 2. LITERATURE REVIEW

The unique flavor and aroma of dairy products has been of concern to consumers for thousands of years (Forss and Sugiwarara, 1981). Since milk itself is a highly perishable product, it was discovered that fermentation could extend the storage life and maintain the nutritive value of the milk supply. These fermentations are primarily responsible for the traditional flavor and aroma associated with fermented dairy products we are familiar with today. Now characterized in great detail, the patterns of aroma-imparting compounds found in fermented dairy products can range in scope from relying on merely a few compounds such as might be found in buttermilk (Lindsay et al., 1966) to potentially hundreds of aroma compounds as have been reported in Cheddar cheese (McGugan, 1975).

Prior to the universal adoption of pasteurization in the 1940's, bacterial contamination of the U.S. milk supply and hence souring of the milk was a most common and pronounced defect. Researchers focusing on the analysis of volatile compounds in dairy products are credited with developing the techniques which involved the isolation and identification of both desirable and undesirable flavor and aroma compounds (Forss and Sugiwarara, 1981). The application of pasteurization dramatically curtailed the spoilage of fluid milk, hence the research focus shifted to investigate the non-enzymatic, non-bacterial derived defects in milk. Chemical tests were

developed to monitor defects, such as lipid oxidation with the thiobarbituric acid test. During the early 1960's the fundamental investigation of those compounds responsible for the flavor and aroma of fermented dairy products was begun.

Of primary importance to fermented dairy products is the development of methods to objectively measure flavor and aroma compounds, and hence aid the prediction of overall quality and consumer acceptability. Criteria for an ideal method would include sensitivity, precision, rapid analysis time, and involve little cost (Vandeweghe and Reineccius, 1990). Our research suggests that the method of solvent desorption dynamic headspace sampling may be such a method.

The following pages discuss solvent desorption dynamic headspace sampling and describe three areas of potential application of solvent desorption dynamic headspace sampling to dairy food aroma issues.

### **SOLVENT DESORPTION DYNAMIC HEADSPACE SAMPLING**

The technique of solvent desorption dynamic headspace (SDD) sampling is a method designed for the analysis of volatile compounds. The process is divided into three major steps: (1) separation of volatile analytes from the food matrix, (2) adsorption and concentration of the analytes onto a trapping device, and (3) desorption and analysis. This general format has

been applied to numerous systems including numerous foods, soil, water and other materials as is described in further detail below.

### **Analyte Separation**

The separation of aroma-imparting analytes from food matrices relies on the volatility of aroma compounds. Volatility is loosely defined as the propensity of a substance to form a vapor (Moeller et al., 1984). An aroma-imparting compound while in the vapor state may enter the nasal passage and be perceived by the olfactory system (Meilgaard et al., 1991). The technique of dynamic headspace sampling relies on the volatile or vapor-forming properties to recover aroma-imparting compounds from food matrices.

In a closed container, volatile compounds exhibiting volatility partition between the food sample and the gaseous headspace above the food, eventually reaching equilibrium under static conditions. The concentration of analyte in the gaseous phase ( $C_a$ ) and food matrix ( $C_f$ ) are constant at equilibrium. The partition coefficient ( $K$ ) is defined as  $C_a/C_f$ .  $K$  can be increased with increasing temperature (Burnett, 1963), added electrolyte (Poll and Flink, 1984), and may be decreased in the presence of binding agents (Thanh et al., 1992). Analytes found in the headspace are no longer bound by food components and are hence available for removal from

the food matrix. Separation is effected by continually purging the sample of food with an appropriate gas. The amount of gas required to obtain a certain percentage recovery can be determined through equations developed through the work of Burnett (1963) and Buttery et al., (1987) and is described in the following section.

### **Purge Gas: Type and Volume Determination**

The selection of purge gas type has not received a great deal of attention. Typical gases employed include air (Buttery et al., 1987) and nitrogen (Vallejo-Cordoba and Nakai, 1993). Nitrogen is most commonly used for dairy foods as a means of preventing oxidative changes (Monnet et al., 1994). For the sake of simplicity, much of this work has been conducted at the standard conditions of 25°C and 760 mm pressure and have been shown to follow theories of volatility based on Henry's law (Buttery et al., 1987).

Henry's law states that the partial pressure of a certain volatile analyte in the headspace above the solution is proportional to the concentration of that analyte in solution. Henry's law can be expressed as:

$$p = C \times N$$

where p is the partial pressure of the analyte above the solution, C is a

constant, and  $N$  is the mole fraction of analyte in solution. The air/water partition coefficient,  $K$ , which follows from Henry's law is defined as:

$$K = \frac{\text{weight of solute per mL of air}}{\text{weight of solute per mL of solution}}$$

The partition coefficient can also be written in terms used for Henry's law:

$$K = p/N \times 0.97 \times 10^{-6}$$

The value  $0.97 \times 10^{-6}$  allows conversion of concentration terms to mole fraction and pressure terms. The volume of gas ( $V_a$ ) required to purge a certain percentage of analyte ( $P$ ) from the sample at 25°C is calculated from

$$V_a = -V_w K^{-1} \ln[(100 - P)/100]$$

where  $V_w$  is the volume of aqueous sample. Hence, if the  $K$  is known or can be calculated, the necessary purge volume can be estimated.

## Adsorption and Concentration of Analytes

As the sample is purged, analytes are removed from the sample, trapped and concentrated on an adsorbent material often referred to as the adsorbent "bed." Adsorbents are classified as Class I, II, or III (Anonymous, 1985).

Class I adsorbents (graphite or graphitized carbon black) are considered nonspecific and contain no ions or active groups (Kiselev and Yashin, 1969). Class I adsorbents are the only class of adsorbents that nonspecifically interact with the four groups of analytes. The four groups include Group A (e.g., n-alkanes), Group B (e.g., aromatic hydrocarbons), Group C (e.g., organometallic compounds), and Group D (e.g., organic acids). Class I adsorbents rely on London dispersion forces to bind airborne analytes (Atkins, 1982). Class II adsorbents (e.g., silica gel) possess localized positive charges and Class III adsorbents (e.g., Tenax® GC) have localized negative charges, and hence, have specific adsorbent/analyte interactions (Betz et al., 1989). A specific Class I adsorbent, Carbotrap™, is also more hydrophobic than other commonly used adsorbents (Anonymous, 1985), thus making its performance as an adsorbent less affected by humidity, an important property since most foods and beverages contain 50 - >95% water making headspace vapors typically very humid.

## **Desorption and Analysis**

Following adsorption and concentration, analytes are removed from the adsorbent bed by exposure to high temperature, reduced pressure or elution with an appropriate solvent (Weurman, 1969). Solvent desorption can be performed by either placing the solvent and adsorbent and solvent in the same vial (Rizzolo et al., 1992) or flushing the sorbent with excess solvent (Buttery et al, 1987). Following desorption, the sample is analyzed via gas chromatography.

Solvent choice greatly affects the recovery and analysis of aroma compounds. A review of typically used solvents is found in Weurman (1969). Solvents are typically glass-distilled just previous to use or purchased in high purity grades. Considerations when choosing a solvent include solvent purity, solubility of analytes in solvent, compatibility with chromatographic column and detector, ease of handling, and toxicity.

## **BUTTERMILK AROMA**

### **Aroma-Imparting Compounds**

Typical, high quality buttermilk aroma is attributed to a delicate blend of organic acids and other volatile compounds produced through the

fermentation of lactose and citrate by a starter culture containing aroma-producing bacteria and acid-producing bacteria (Lindsay et al., 1966). The acid-producing bacteria, typically *Lactococcus cremoris*, ferment lactose into primarily lactic acid with lesser amounts of acetic, propionic, formic, and pyruvic acids and trace amounts of carbon dioxide, acetaldehyde, and ethanol (Marsili, 1985). Aroma-producing bacteria, such as *Leuconostoc citrovorum*, ferment citrate into diacetyl, the major desirable aroma-imparting component in buttermilk (Collins, 1971).

Diacetyl has been of interest to the dairy researcher for many years. Almost synonymous with the term "dairy aroma," diacetyl imparts an aroma associated with heat treated butter. The aroma threshold of diacetyl (minimum perceptible concentration) ranges from 0.02 to 0.1 parts per million (ppm) in milk (Bennett et al., 1964; Hempenius et al., 1966). Desirable concentrations of diacetyl in buttermilk have been reported to be in the range of 2 - 4 ppm (Lindsay et al., 1966). Diacetyl levels that are too high result in a harsh, overbearing aroma. Conversely, buttermilk with low levels of diacetyl is criticized as being "flat" or having little or no cultured flavor.

Low levels of diacetyl may result from low levels of fermentable citrate in the milk, but are most likely due to the presence of the enzyme diacetyl reductase. Elliker (1945) demonstrated the effect of numerous bacteria on the reduction of diacetyl to acetoin and 2,3-butanediol. Early work suggests that acetoin and 2,3-butanediol make no contribution to the aroma.

Recently, reports suggest that acetoin may have a unique aroma character (Christensen and Reineccius, 1995; Arora et al., 1995). Seitz et al. (1963) showed that many starter cultures have high levels of diacetyl reductase. In both of these studies and in many others, psychotrophic bacteria, namely *Pseudomonas* spp, exhibited high levels of diacetyl reductase activity. It is generally recognized that psychotrophic bacteria from post-process contamination are the main source of diacetyl reductase in fermented dairy products.

### **Objective Analysis of Buttermilk Aroma**

Several methods exist for the determination of diacetyl and other volatile compounds in buttermilk. These methods can be classified into one of two broad categories: chemical or chromatographic methods.

Nearly all of the chemical methods for the determination of diacetyl in biological materials are based upon the work described by Westerfeld (1945) which relies on the formation of insoluble nickel dimethylglyoxime when diacetyl is treated with hydroxylamine. Owades and Jakovac (1963) and Pack et al. (1964) applied this earlier work to the determination of diacetyl in beer and in dairy systems, respectively. In their methods, diacetyl is either steam distilled or purged from the sample with nitrogen gas. Diacetyl is then trapped in a small aliquot of buffered hydroxyamine, then converted to

dimethyl glyoxime. Dimethyl glyoxime produces a pink color which is read by absorbance at 530 nm.

Acetoin assays were conducted by first determining the concentration of diacetyl by the above method. A fresh sample was then treated with ferrous sulfate to oxidize acetoin to diacetyl. The sample is then assayed again for diacetyl and the difference between the iron-treated and untreated sample is calculated as the amount of acetoin.

Chromatographic methods rely on the separation of diacetyl from the food matrix and quantification of diacetyl through traditional chromatographic methods. These methods relied on the separation of diacetyl from the food matrix by either solvent extraction (Lee and Drucker, 1975), static headspace sampling (Marsili, 1985) or a purge and trap method (Kang et al., 1988). A variety of column types have been shown in the literature to provide sufficient resolution, with polyethylene glycol columns being most commonly used. Although flame ionization detectors are typically used, electron capture detectors were shown to be much more sensitive (Scanlan and Lindsay, 1968; Otsuka and Ohmori, 1992).

## STABILIZING AND EMULSIFYING AGENTS IN DAIRY FOODS

### **Typically Employed Dairy Food Stabilizers and Emulsifiers**

Both stabilizers and emulsifiers have found use in dairy foods where control of body and texture characteristics is important. Trends towards reduced-fat products have increased the use of stabilizing and emulsifying agents in an attempt to provide fat mimetic properties.

Stabilizers and emulsifiers in dairy products serve several purposes some of which include thickening (Whistler and BeMiller, 1993), protein stabilization (Lin, 1977), inhibition of cream separation (Therkelsen, 1993), and creation of oil-in-water and water-in-oil emulsions. The following is a discussion of typically used stabilizers and emulsifiers used in the dairy industry.

Carrageenan is derived from seaweed of the red algal species, *Chondrus crispus*, and is thought to have been first used in Ireland, hence the name Irish moss. Carrageenan is characterized as a group of sulfated galactans (Sand and Glicksman, 1973) which are the major structural polysaccharides in red algae (30 - 80%, dry basis). Typical carrageenan contains three major fractions,  $\kappa$ ,  $\iota$ , and  $\lambda$ -carrageenan and four minor fractions,  $\mu$ ,  $\nu$ ,  $\theta$ , and  $\xi$ . Variation among these fractions results primarily from differences in position and number of sulfate groups. Carrageenan is

widely used in dairy foods and a review of typical applications is found in Therkelsen (1993).

Guar gum is derived from the seed of *Cyamopsis tetragonolbus*, a legume. The functional polysaccharide in guar gum, guaran, is constituted of (1 → 4)-linked β-D-mannopyranosyl units with a single α-D-galactopyranosyl unit connected by (1 → 6) linkages to approximately every second main chain unit (Maier et al., 1993). It is used in combination with other hydrocolloids such as carrageenan and locust bean gum. Guar gum is used in cold-pack cheese, sour creams and other dairy products where hydration properties and acid stability are important.

Produced by *Xanthamonas campestris*, xanthan gum was one of the first microbial polysaccharides employed for commercial use. Xanthan gum has been studied extensively for toxicity and was granted FDA approval in 1969 without any specific quantity limitations. FDA regulations permit the use of xanthan gum in numerous milk and cream products. The structural backbone of xanthan gum is identical to that of cellulose, (1 → 4)-linked β-D-glucopyranosyl units. Alternate glucosyl units have attached a trisaccharide consisting of D-glucuronosyl unit between two D-mannosyl units. The terminal D-mannosyl unit is glycosidically linked to an α-D-mannopyranosyl unit. Approximately 50% of the the terminal D-mannosyl units have a pyruvic acid moiety as a 4,6-cyclic acetal (Kang and Pettitt, 1993).

Xanthan gum is widely used in the food and dairy industry for numerous reasons including high viscosity at low concentrations, acid stability, and compatibility with salts. Specifically, xanthan gum is used in cottage cheese dressings to prevent whey-off and improves cling of dressing to the curd. It is commonly used in combination with guar gum and locust bean gum to improve the properties of cheese spreads and numerous milk and cream products.

Polysorbates or polyethylene sorbitan esters are formed from the reaction of sorbitan esters with ethylene oxide. Typically esters are formed with either stearic acid or oleic acid. Polysorbates provide emulsion stability, improve gloss, and firmness to dairy products (Dziezak, 1988).

Lecithin is a blend of phosphatides that contain phosphatidyl choline, phosphatidyl ethanolamines, and inositol phosphatides and other components. Commercially used lecithin is derived from soy beans with a smaller portion coming from egg yolk. Lecithin promotes oil in water emulsions and is used in ice cream and cream dressings (Nawar, 1985).

Carboxymethyl cellulose (CMC) serves many functions as a food additive. It is manufactured by the treatment of cellulose with sodium hydroxide-chloroacetic acid. An ether linkage is formed to the acetic acid moiety with a degree of substitution of 0.7 - 1.0. CMC forms soluble salts with monovalent cations and hazy dispersion with divalent cations. CMC can act

to stabilize proteins in solution, stabilize emulsions, and improve mouthfeel (Whistler and Daniel, 1985).

### **Interaction of Volatile Compounds with Food Components**

Almost all foods exist as complex, non-uniform mixtures of food components. The perceived aroma of foods can be influenced by the chemical and physical contributions of these components. Several studies have shown that many volatile compounds interact with other food components. The interaction between volatiles and other food components may decrease the concentration of volatiles in headspace vapors, hence have the potential to decrease the sensory impact of aroma-imparting compounds.

Ahmed et al. (1978) determined that the flavor threshold of d-limonene is reduced through interaction with volatile juice constituents. Thanh et al. (1992) suggests that interactions between volatile compounds and other food components result from a variety of bonds including hydrophobic interactions, hydrogen bonding, ionic interactions, and covalent bonding. Saleeb and Pickup (1978) calculated the heats of adsorption of several volatile compounds under equilibrium conditions. However, during consumption of foods, sensory perception is affected by the extent and rate of release of volatiles in a non-equilibrium state. Hence, the interaction

of volatile compounds with other food components would be best studied under non-equilibrium, dynamic conditions.

## CHEDDAR CHEESE AROMA AND FLAVOR

### **Aroma-Imparting Compounds**

Early work on the flavor of Cheddar cheese was based on the hypothesis that there was only one compound or one class of compounds that produced typical Cheddar flavor. However, since researchers were unable to prove that hypothesis, Kosikowski and Mocquot (1958) proposed the component balance theory of Cheddar cheese flavor. They proposed that Cheddar flavor is produced by the sensory perception of several compounds that, in the right concentrations, elicits desirable Cheddar flavor. Current research on Cheddar flavor is still based on the component balance theory.

More than 200 different volatile compounds have been identified in Cheddar cheese and several reviews of Cheddar aroma are available (Fors and Patton, 1966; McGugan, 1975). Current research has focused on determining which of the over 200 volatile compounds recovered from Cheddar cheese actually contribute to the aroma.

Higher boiling compounds have also been examined through the use of distillation (Day and Libbey, 1964; Dunn and Lindsay, 1985), acetonitrile

extracts (Jolly and Kosikowski, 1975), HPLC, and headspace concentration (Dunn and Lindsay, 1985). These methods are adequate for the determination of high boiling compounds such as acetoin, C<sub>4</sub> - C<sub>14</sub> fatty acids and various lactone compounds. Although the compounds isolated in these methods may have some impact on the overall flavor of Cheddar cheese, it is proposed that the lower boiling, more volatile compounds may be most critical for aroma (Manning and Robinson, 1973). Although hundreds of compounds are recoverable from Cheddar cheese, perhaps only a fraction of those compounds contributes to the aroma of mature Cheddar cheese.

Dacremont and Vickers (1994) examined a mineral oil model system with a trained sensory panel to screen 15 compounds suspected to contribute to the flavor of Cheddar cheese. While acetoin was found to moderately increase Cheddar-like flavor, their findings suggest that the most important compounds for desirable flavor were butyric acid (16 to 40 ppm), diacetyl (20 ppm), and methional (0.8 ppm). Keen and Walker (1974) found that diacetyl was detectable in cheese only during the first five months of aging.

Christensen and Reineccius (1995) used Aroma Extraction Dilution Analysis to determine which volatile compounds were the most potent contributors to Cheddar cheese aroma. Cheddar cheese oil was subjected to molecular distillation; volatile compounds were recovered and a stock of concentrated aroma compounds was made in diethyl ether. A series of dilutions (1:3) of the aroma stock were made and then evaluated with GC

effluent sniff port analysis. Compounds with highest potency (concentration to aroma threshold) were butyric acid, ethyl caproate, acetic acid with lesser potencies for ethyl butyrate, acetoin, and valeric acid. A possible contribution from 1-octen-3-one was also mentioned although its identity in their study is considered tentative. Interestingly, the sensory differences between Camembert and Brie cheeses may be due in part to the presence of 1-octen-3-ol in the former (Forss and Sugiwarara, 1981).

Of primary concern and importance among the lipid-derived aroma compounds are the lower molecular weight free fatty acids (FFA). These FFA result from the enzymatic hydrolysis of milk triglyceride by bacterial or endogenous lipase activity. The fatty acid composition of milk fat is unique due to the presence of short-chain fatty acids which, being volatile, impart potent and distinct character to milk products.

Although studies have shown that lipase activity results in the liberation of all milk fat fatty acids, only the low molecular weight fatty acids (C<sub>2-10</sub>) are thought to contribute to aroma (Patton, 1963). Most synthetic cheese flavors evaluated contain FFAs (Day et al., 1960; Walker, 1961). It is well accepted that milk fat plays a vital role in Cheddar cheese flavor development (Dean, 1972; Dean and Dolan, 1973). Foda et al. (1971, 1974) suggest that the principal role of milk fat is to dissolve and act as a carrier for flavor and aroma compounds.

The peptides and amino acids resulting from hydrolysis of proteins in dairy products are primarily non-volatile, hence are not aroma-imparting (Forss and Sugiwarara, 1981). However, certain peptides may contribute to bitterness, sourness and sweetness. Proteins are also the primary source of sulfur compounds in dairy products.

Sulfur containing compounds isolated from Cheddar cheese include hydrogen sulfide, methanethiol, dimethyl sulfide (Manning and Robinson, 1973), dimethyl disulfide (Horwood, 1989), ethyl mercaptan (Liebach et al., 1970), methyl mercaptan (Libbey and Day, 1963), and methional (Keeney and Day, 1957). Sulfur compounds are considered of importance in Cheddar flavor due to their low aroma threshold and distinctive aroma character. Dacremont and Vickers (1994) suggest that methional is of primary importance to Cheddar aroma. However, methional degrades easily into dimethyl sulfide and methyl mercaptan (Forss and Patton, 1966) which are both commonly identified in Cheddar aroma concentrates. Kristoffersen and Gould (1960) and Walker (1961) propose that hydrogen sulfide is important in Cheddar aroma although its concentration does not change with aging.

The formation of  $\gamma$ - and  $\delta$ -lactones arise from the thermal breakdown of  $\gamma$ - and  $\delta$ - hydroxyacids (Forss, 1972). The C<sub>8</sub> 10 12  $\delta$ -lactones contribute to the flavor of butter and heated foods containing butter. O'Keefe et al. (1969)

isolated a "coconut" aroma fraction composed primarily of a series of  $\gamma$ - and  $\delta$ -lactones.

Dacre (1955) proposed that ethyl esters have little effect on the aroma of typical Cheddar cheese. Bills et al., (1965) reported that ethyl butyrate and ethyl caproate are associated with the fruity flavor defect in Cheddar cheese.

### **Techniques for the Identification of Aroma Compounds in Cheddar Cheese**

A clear understanding of the origin and composition of Cheddar cheese would act as an objective predictor of overall quality and aid in the assessment of sensory defects. However, in spite of some 50 years of research, the origin and composition of Cheddar cheese aroma have escaped clear definition.

The preparation of an isolate suitable for gas chromatographic analysis has been a challenge to the researcher and numerous methods of preparation exist. General methods for the analysis of volatile compounds in food products are found in Weurman (1969) and Teranishi et al., (1971). Some of these methods include steam distillation (Aishima and Nakai, 1987), direct injection of Cheddar cheese oil (Liebich et al., 1970), dialysis (Benkler and Reineccius, 1980), molecular distillation (Scarpellino and Kosikowski, 1961), solvent extraction (Wong and Park, 1968), and headspace sampling (Horwood, 1989). Although each of these methods are able to

identify numerous compounds in Cheddar cheese, no one method provides satisfactory analysis of all aroma compounds.

It was proposed by McGugan et al. (1979) that methods relying on the separation of fat results in considerable losses of water soluble compounds. However, methods such as these are still in use. Molecular distillation to trap volatiles from Cheddar cheese oil has been used both in the past (Libbey et al., 1963) and recently (Christensen and Reineccius, 1995).

A recent comparison of Cheddar cheese flavor isolation techniques was conducted by Vandeweghe and Reineccius (1990). They demonstrated that solvent extraction gave the least concentrated isolate with the most Cheddar-like aroma. The dialysis method was most concentrated, but was costly and each isolate took three days to prepare.

### SUMMARY

This literature review has described major components germane to the research conducted. First reviewed was a description of a simple method for the analysis of volatile compounds in food matrices. The next sections described three issues relevant to the dairy foods industry, namely aroma assessment in buttermilk, inhibition of diacetyl release by stabilizing and emulsifying agents, and the development of a rapid method for the analysis of Cheddar cheese aroma. The focus and intent of research conducted for this

dissertation was to evaluate the performance of solvent desorption dynamic headspace sampling as applied to these three issues. It is hoped that the simple, rapid methods such as described and examined in this dissertation will contribute to the development of routine analyses for numerous aroma-related issues in dairy products as well as other foods.

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## CHAPTER 3

### SOLVENT DESORPTION DYNAMIC HEADSPACE METHOD FOR DIACETYL AND ACETOIN IN BUTTERMILK\*

Scott A. Rankin and Floyd W. Bodyfelt

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### ABSTRACT

Major buttermilk volatiles recovered included diacetyl, acetic acid, and acetoin. Detection limits were diacetyl 0.2 and acetoin 2.0  $\mu\text{g/g}$ . Mean percent recoveries were 112% for diacetyl and 8.08% for acetoin. Normalized detector responses were linear over the range of concentrations tested ( $R^2 > 0.999$ ) for diacetyl and acetoin. Percent relative standard deviations from quadruplicate analysis of 7-day-old buttermilk were  $< 8\%$  for diacetyl, acetoin, and acetic acid. The method enabled quantitative estimation of diacetyl and acetoin in  $< 30$  min, including sample preparation time.

## INTRODUCTION

Fermentation and flavor development in buttermilk occur through the action of a mixed starter culture that contains both acid- and aroma-producing bacteria. The aroma-producing bacteria are responsible for fermentation of citrate into diacetyl which imparts the desirable buttery aroma. Diacetyl is reduced in the presence of diacetyl reductase into acetoin (Collins, 1971), resulting in a substantial loss of desirable aroma. The presence and quantity of acetoin is indicative of diacetyl reductase which can be found in starter cultures (Kneifel et al., 1992) and is commonly associated with psychrotrophic bacteria (Seitz et al., 1963).

The assessment of diacetyl, acetoin, and other compounds associated with the flavor and aroma of commercial buttermilk has been the subject of many research reports. Typical assays include colorimetry (Westerfeld, 1945; Pack et al., 1964; Walsh and Cogan, 1974), chemical derivatization (Damiani and Burini, 1988; Martineau et al., 1994), solvent extraction (Jansen et al., 1979), static headspace sampling (Ulberth, 1991), and thermal desorption dynamic headspace (TDD) sampling (Yang and Min, 1994). Each method has inherent limitations.

Static headspace sampling typically is not sensitive enough to directly assay for acetoin (Marsili, 1981; Ulberth, 1991). Thermal desorption dynamic headspace analysis can detect many volatile compounds including diacetyl and acetoin (Laye et al., 1993). However, the high cost of thermal desorption

and cryogenic focusing equipment has been prohibitive. Also, storage of static and dynamic headspace samples is impossible since the entire sample is injected at one time. Most of the methods involve heating, which can promote spontaneous breakdown of  $\alpha$ -acetolactic acid into diacetyl (Jönsson and Pettersson, 1977), resulting in overestimation of diacetyl.

Solvent desorption dynamic (SDD) headspace sampling is a simple technique commonly employed for many volatile compounds (Olafsdottir et al., 1985; Rizzolo et al., 1992). However, few reports have been made concerning SDD to determine aroma compounds in fermented dairy products (Kang et al., 1988).

Our objective was to characterize the effectiveness of a low cost, commercially available solvent desorption trap to quantitatively measure diacetyl and acetoin in buttermilk.

## MATERIALS & METHODS

### Model System

The ability of the SDD method to detect and quantify diacetyl and acetoin was examined with a fermented milk model system similar to that of Ulberth (1991). The model system consisted of commercially pasteurized, homogenized milk (2% fat) with varying concentrations of added diacetyl (0 - 32  $\mu\text{g/g}$ ) and acetoin (0 - 512  $\mu\text{g/g}$ ) standards. Lactic acid (J.T. Baker Inc., Phillipsburg, NJ) was added to simulate the pH (4.5) and provide the

consistency of a fermented milk product. Diacetyl, acetoin, 2-butanone, acetic acid and 1-propanol were purchased from Sigma-Aldrich (St. Louis, MO). Standard purity was determined with preliminary gas chromatographic (GC) analysis. The model system was used to establish retention times of target compounds, develop calibration curves, determine % recovery and % relative standard deviation (RSD) and to estimate limits of detection (LOD) for diacetyl and acetoin.

### **Buttermilk Samples**

Containers (946 mL) of fiberboard-packaged buttermilk were obtained from Lochmead Dairy (Junction City, OR) on the day of manufacture and stored at 4°C. Duplicate cartons were randomly selected for sampling at 3-day intervals. RSD's for quantifiable aroma compounds were determined by quadruplicate analysis of buttermilk samples at 7 days storage.

### **Dynamic Headspace Analysis**

A 10-g sample was placed in a 40-mL glass vial and sealed with a screw-top Teflon-lined cap. A 100- $\mu$ L aliquot of 1-propanol internal standard solution (5  $\mu$ g/g) was added to each vial prior to homogenization with a vortex mixer. A 3-g sample was transferred from the glass vial into a 5-mL round-bottom flask with a universal inlet adapter.

Sample purge was conducted by immersing the flask in a  $30 \pm 1^\circ\text{C}$  circulating water bath. A submersible stirrer on highest setting and Teflon starburst stirring head (9.5 mm, Fisher Scientific, Pittsburgh, PA) were used to provide thorough agitation of samples, facilitate heat transfer, and increase gas/liquid interaction. Nitrogen was the purge gas to limit oxidative changes (Monnet et al., 1994). Purge gas was passed through a moisture and hydrocarbon trap (Restek, Bellefonte, PA). Teflon tubing was used for all connections following the gas filters. Purge was conducted by swept surface (Kang et al., 1988) to avoid foaming and inclusion of antifoam agents. Purge volume ( $V_a$ ) was estimated for 99% recovery (P) of diacetyl using the relationship

$$V_a = -V_w K^{-1} \ln[(100 - P)/100]$$

where K is the air/water partition coefficient of diacetyl at  $25^\circ\text{C}$  and  $V_w$  is the volume of aqueous solution (Buttery et al., 1987). The K for diacetyl was computed using the equation (Buttery et al., 1971)

$$K = p/N \times 0.97 \times 10^{-6}$$

where p is the vapor pressure of pure diacetyl at  $25^\circ\text{C}$  (Lidl, 1991) and N is the

solubility in water expressed as a mole fraction (Thanh et al., 1992). Thus,

$$K = 55.88 / 0.5233 \times 0.97 \times 10^{-6} = 1.036 \times 10^{-3}$$

A rate of 800 mL/min was the minimum purge rate to prevent visible condensation of water in the adsorbent bed. The final calculated purge volume was 16 L.

Commercially available Carboxen traps (ORBO 90, Supelco, Bellefonte, PA) were used because they trap carbonyl compounds, are hydrophobic, and are relatively chemically inert (Betz et al., 1989). Following sample purge, traps were immediately eluted with GC/MS grade acetone (Sigma-Aldrich, St. Louis, MO). The first mL of solvent eluate was collected in a 1.0-mL volumetric flask. Eluting with this volume resulted in complete desorption of diacetyl and acetoin from the sorbent material. The eluate was then transferred to a 2-mL glass vial with Teflon-lined cap. A new trap was used for each analysis. Traps were examined for analyte bleed by placing the breakthrough section of the trap in a 2-mL glass vial, eluting with ~100  $\mu$ L of acetone, and conducting GC analysis for volatile compounds.

### **Separation and Identification of Volatiles**

Identities of diacetyl, acetic acid and acetoin were determined by retention time of injected standards and were confirmed with mass spectroscopy. GC was carried out with a Hewlett Packard (HP, Avondale, PA)

5890 gas chromatograph with flame ionization detector (FID) under the following conditions. Initial temperature was 35°C isothermic for 7 min, increased at 20°C/min to 120°C, then increased at 40°C/min to 200°C and maintained for 5 min. A Restek (Bellefonte, PA) Rtx-624 column was employed to separate the compounds (30 m, 0.32 mm i.d., 1.8 µm film thickness). Column flow rate was 1.5 mL/min. Sample size was 1 µL, splitless injection. An HP 3396 integrator was used for peak area determination.

Mass spectroscopy was performed with a Varian 3400 (Palo Alto, CA) mass spectrometer with column and chromatographic conditions as described. Conditions were ion source temperature, 170°C; ionization voltage 70 eV; mass scan range, m/e 20 - 200; scan rate, 1.0 scan/sec. Compounds were tentatively identified in buttermilk by comparison of sample spectra with library reference spectra and further confirmed by comparing mass spectra and GC retention times to known standards.

## RESULTS & DISCUSSION

### Method Development

It is possible to adjust method parameters to improve sensitivity. A target LOD of 0.2 µg/g for diacetyl was established since it is the sensory threshold of diacetyl in acidified milk (Bennett et al., 1964). The LOD for acetoin was 2.0 µg/g. Initially, solvent desorption was performed by placing

the purge tube sorbent into a 2-mL vial followed by addition of 300  $\mu$ L CS<sub>2</sub>. However, upon storage diacetyl levels decreased by 40%. Analysis of samples with markedly decreased levels of diacetyl showed no changes in levels of other analytes and no potential diacetyl breakdown products. Diacetyl loss was minimized by eluting the trap with solvent and collecting the sample in the absence of sorbent material.

Although CS<sub>2</sub> is typically the solvent used for SDD, acetoin is relatively insoluble in CS<sub>2</sub>. Acetone was used because it dissolves both diacetyl and acetoin, is stable, safe for handling, and available in relatively pure form. Although the acetone we used had been glass distilled by the manufacturer, several contaminants were detected. Since these had different retention times and did not interfere with target compound peaks, no attempts were made to further purify the acetone.

### **Model System Analysis**

Normalized FID response to added amounts of diacetyl and acetoin exhibited good linearity ( $R^2 > 0.999$ ) over the concentrations tested. As predicted, recovery of diacetyl was high (Table 3.1). Continued sampling ( $V_a > 16$  L) resulted in no increased diacetyl levels. Recovery of acetoin averaged <10% for all concentrations tested. Although low recovery has been reported to result in poor reproducibility (Westerndorf, 1985), RSD's for acetoin were considered acceptable. Quantification of acetoin in unknown

samples depends on calibration curves that correct for the % recovery or could be reported in relative amounts for treatment comparisons.

**Table 3.1- Precision and accuracy of diacetyl and acetoin estimation in fermented milk model system.**

diacetyl				acetoin				
expected <sup>a</sup>	found	%recovery	%RSD <sup>b</sup>	expected	found	predicted	%recovery	%RSD
0	ND <sup>c</sup>	-	-	0	ND	-	-	-
1	1.16	115	3.00	1	ND	-	-	-
2	2.05	102	1.38	2	0.020	2.14	1.00	165
4	4.50	112	7.32	4	0.285	4.80	7.12	10.9
8	9.31	116	0.95	8	0.633	8.30	7.92	4.26
16	17.38	108	3.55	16	1.36	15.6	8.41	4.15
32	37.34	116	0.55	32	3.00	32.1	9.36	2.58
				64	6.15	63.8	9.61	9.53
				128	11.7	120	9.17	3.79
				256	26.4	270	10.3	5.26
				512	50.4	509	9.84	0.782

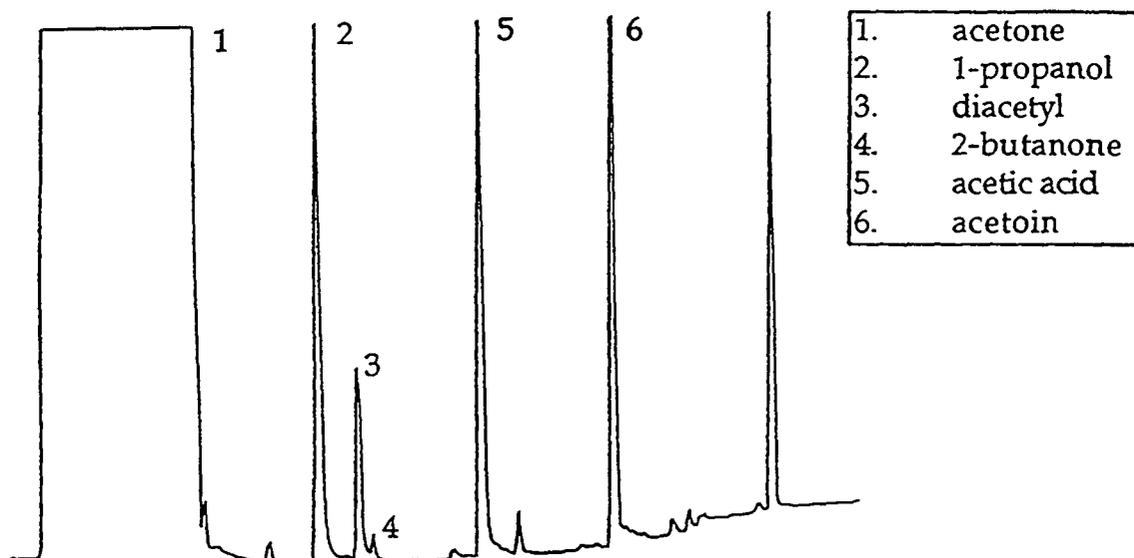
<sup>a</sup> µg/g.

<sup>b</sup> % relative standard deviation from triplicate analyses.

<sup>c</sup> ND = not detected. These results would be expected given the relative difference in boiling points (88 vs. 147°C) and air/water partition coefficients of diacetyl and acetoin.

### Buttermilk Analysis

Several compounds were identified in buttermilk samples (Fig. 3.1). Quantification of ethanol and acetaldehyde was impossible with the described purge parameters. Both coeluted with an acetone solvent contaminant peak and exhibited sorbent bed breakthrough. No breakthrough of diacetyl, acetoin, acetic acid, or 2-butanone was indicated.



**Figure 3.1.** Gas chromatogram of volatile compounds from buttermilk. Separations on a 6% cyanopropylphenyl, 94% methyl silicone fused silica capillary column (30 m  $\times$  0.32 mm i.d.  $\times$  1.8  $\mu$ m film thickness). Unidentified peaks are solvent contaminants.

Method precision for quantifiable aroma-contributing compounds in buttermilk was highest for diacetyl and least for acetic acid (Table 3.2).

**Table 3.2- Gas chromatographic detection of buttermilk aroma volatiles.**

compound	RT <sup>a</sup>	RSD <sup>b</sup>
acetone	6.51	ND <sup>d</sup>
1-propanol <sup>c</sup>	8.83	ND
diacetyl	9.42	3.21
2-butanone	9.61	ND
acetic acid	11.13	7.08
acetoin	12.75	6.36

<sup>a</sup> RT = retention time in min with Rtx-624 30 m capillary column.

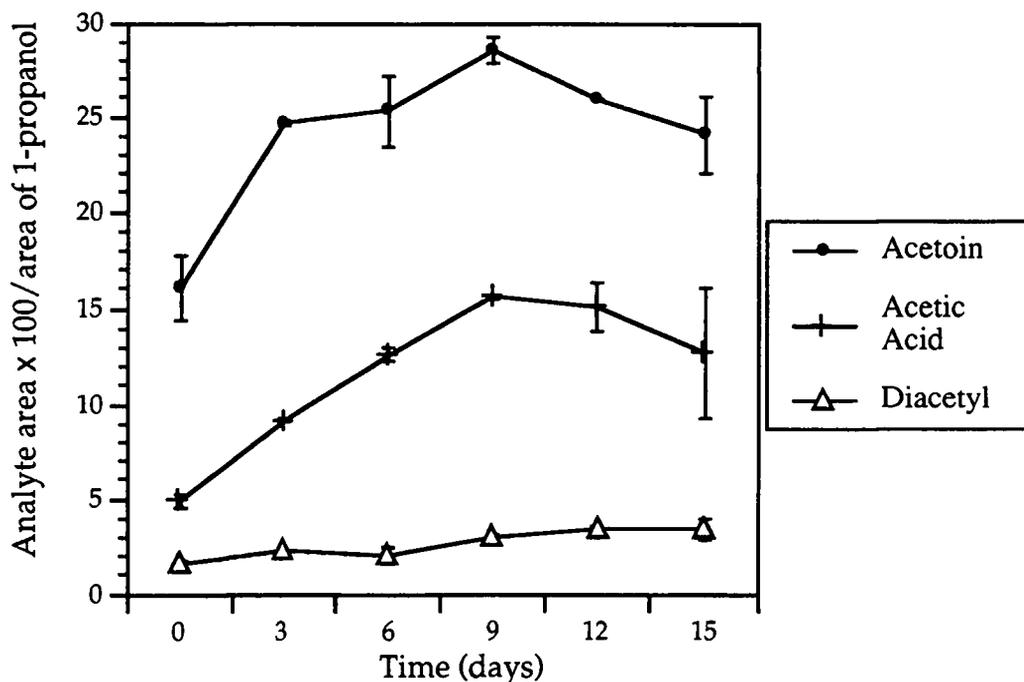
<sup>b</sup> %RSD = % relative standard deviation based on quadruplicate analysis of 7-day-old buttermilk samples.

<sup>c</sup> internal standard.

<sup>d</sup> ND = not determined.

However, all RSD's were considered quite low and related well to reported values (Ulberth, 1991; Monnet et al., 1994).

Aroma profiles of buttermilk samples analyzed over 15 days storage at 4°C showed changes (Marsili, 1981; Kang et al., 1988; Monnet et al., 1994) typical of high-quality fermented milk (Fig. 3.2). All compounds reached maximum levels at 9 - 12 days storage and then diminished in concentration.



**Figure 3.2.** Profiles of diacetyl ( $\Delta$ ), acetic acid (+) and acetoin ( $\bullet$ ) in buttermilk samples over 15 days storage at 4°C. Error bars represent standard deviations from duplicate analysis.

## CONCLUSIONS

Using commercially available, low cost, preconditioned adsorbent traps resulted in a rapid, convenient method for estimating diacetyl and acetoin. Headspace sampling parameters and solvent choice could be changed to optimize recovery and quantification of other aroma compounds. This method is superior to colorimetric tests commonly employed as it suffers less from chemical interference and is capable of quantifying several

volatile compounds simultaneously. Other advantages include minimal sample preparation, low heat treatment, modest equipment investment, and rapid sample analysis.

### ACKNOWLEDGEMENTS

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**CHAPTER 4****EFFECT OF STABILIZERS AND EMULSIFIERS ON HEADSPACE  
AVAILABLE DIACETYL IN A MODEL DAIRY SYSTEM\***

Scott A. Rankin and Floyd W. Bodyfelt

\*This chapter was submitted to the Journal of Food Science (Oct., 1995) and is in press.

## ABSTRACT

Three commercially used stabilizers (guar, xanthan, and carrageenan) and emulsifiers (lecithin, carboxymethyl cellulose, and Tween 80), were examined for their effects on headspace available diacetyl at 0.05, 0.10, and 0.20% (wt/wt) in a 5% milkfat model system. All additives inhibited the release of diacetyl on the average of 40% as compared to a control. In general, headspace available diacetyl decreased with increasing system viscosity. Guar gum and carrageenan exhibited similar diacetyl release inhibition when corrected for viscosity. Xanthan gum exhibited the greatest decrease in headspace available diacetyl after correction for viscosity at increasing gum levels. Tween 80 imparted no significant viscosity and had no effect on recoverable diacetyl. Lecithin had no effect on viscosity, however it did inhibit the release of diacetyl as a function of lecithin level. Carboxymethyl cellulose increased viscosity and inhibited diacetyl release.

## INTRODUCTION

Dairy products such as cottage cheese, yogurt and sour cream increasingly rely on added stabilizing and emulsifying agents to impart desirable texture and appearance characteristics. Recent increased consumer demand for reduced fat dairy products has also necessitated widespread use of these agents as fat mimetics in product formulations. While these additives impart desirable properties to foods, studies have shown that these compounds can bind or inhibit the release of aroma-imparting compounds (Dumont and Land, 1986; Thahn et al., 1992).

Nonvolatile food components such as protein and carbohydrates may either increase or decrease the volatility of flavor-imparting compounds. Hansen and Heinis (1992) demonstrated that casein and whey proteins decrease sensory perception of several aroma compounds associated with citrus flavors. Thanh et al (1992) showed that certain polysaccharides have the potential to bind a variety of flavor imparting compounds including diacetyl. The binding phenomenon with diacetyl may result from covalent bond formation (Schiff's base), hydrogen bonding, hydrophobic interaction, or the formation of inclusion complexes (Hansen and Heinis, 1992; Solms et al., 1973; Thahn et al., 1992).

Analytical methods used to examine the effect of binding are typically conducted at equilibrium conditions (O'Keefe et al., 1991). While it may be of analytical interest to examine aroma binding at equilibrium, it may be

argued that since consumption of food does not occur under equilibrium conditions, a dynamic headspace recovery system would be more appropriate to examine the potential inhibition effect.

The desirable flavor in many fermented dairy products relies on the presence and availability of volatile aromatic compounds such as diacetyl. However, little work has been conducted to examine the effect of commercially used stabilizers and emulsifiers on the inhibition of diacetyl release (Bennett et al., 1964). The objective of this study was to determine the effect of stabilizer and emulsifier type, level, and resulting viscosity on the release of diacetyl using dynamic headspace sampling.

## **MATERIALS & METHODS**

### **Model System**

The model dairy system was based on a 5% milk fat commercially homogenized, pasteurized milk. Babcock analyses were conducted to confirm milkfat content. Stabilizers (guar, xanthan, and carrageenan (type I, commercial grade)) and emulsifiers (polyoxyethylenesorbitan monooleate (Tween 80), carboxymethyl cellulose, sodium salt (medium viscosity) were purchased from Sigma-Aldrich (St. Louis, MO). Lecithin (soybean, oil removed) was purchased from ICN Biomedicals (Costa Mesa, AZ). Aliquots of 150 mL of milk were placed into 250 mL glass vials with Teflon-lined screw-top lids. Dry stabilizers and emulsifiers were dispersed into the milk by

first adding the dry powder to nonfat dry milk powder (Familiar Foods, Inc., City of Industry, CA.; total 1.0 g added solids) under constant agitation with a magnetic stirrer. Tween 80 was prepared as an aqueous solution. Samples were allowed to rehydrate overnight at 4°C. The following day, treatments were batch pasteurized in a 75°C circulating water bath for 25 min. The samples were immediately cooled in an ice bath to 30°C. A 100 µL aliquot of diacetyl solution (final concentration 4 µg/g, Sigma-Aldrich, St. Louis, MO) was added to each vial then stored overnight at 4°C until analysis.

Diacetyl purity was determined with gas chromatography. Although minor contaminant peaks existed, the diacetyl stock was >99.9% pure, hence no attempts were made to further purify the sample.

### **Dynamic Headspace Analysis**

A 3-g aliquot of treatment sample was transferred into a 5-mL round-bottom flask fitted with a universal inlet adapter. Sample purge was conducted by immersing the flask in a  $4 \pm 1^\circ\text{C}$  circulating water bath. A submersible stirrer on lowest setting and Teflon starburst stirring head (3/8 in, Fisher Scientific, Pittsburgh, PA) were used to provide thorough agitation of samples. Nitrogen was chosen as the purge gas to limit oxidative changes (Monnet et al., 1994). Purge gas was passed through a moisture and hydrocarbon trap (Restek, Bellefonte, PA) . Teflon tubing was used for all connections following the gas filters. Purge was conducted in a swept surface

orientation (Kang et al., 1988) to avoid foaming and inclusion of antifoam agents. Samples were purged at 400 mL/min for 15 min; the final purge volume was 6.0 L.

Commercially available Carboxen traps (ORBO 90, Supelco, Bellefonte, PA) were selected for their ability to trap diacetyl (Rankin and Bodyfelt, 1995). A new trap was used for each analysis. Following sample purge, traps were immediately eluted with GC/MS grade acetone (Sigma-Aldrich, St. Louis, MO). The first mL of solvent eluate was collected in a 1.0-mL volumetric flask then transferred to a 2-mL glass vial with Teflon-lined cap. Following elution, 100  $\mu$ l of aqueous 1-propanol (Sigma-Aldrich, St. Louis, MO) external standard was added to correct for injection volume error. Quantitation was based on results from injection of samples of known diacetyl concentration.

### **Gas Chromatography**

The identity of diacetyl was determined by retention time of an injected standard. Gas chromatography was carried out with a Hewlett Packard (HP, Avondale, PA) 5890 gas chromatograph with flame ionization detector (FID) under the following conditions. Initial temperature was 35°C isothermic for 7 min; increased by 20°C/min to 120°C; then increased at 40°C/min to 200°C and maintained for 5 min. A Restek (Bellefonte, PA) Rtx-624 column was employed (30 m, 0.32 mm i.d., 1.8  $\mu$ m film thickness).

Column flow rate was 1.5 mL/min and sample size was 1  $\mu$ L, splitless injection. An HP 3396 integrator was used for peak area determination.

### **Viscosity Measurement**

Viscosity was determined using a Brookfield model RVT viscometer. Samples were randomly selected from storage at 4°C for viscosity measurement in triplicate. Log transformations of results were performed to normalize viscosity data for statistical analysis.

### **Data Analysis**

A 3  $\times$  3 factorial experiment was designed with viscosity treated as a covariate. GLM was conducted using SAS (1985) with the following model:

$$\text{diacetyl} = \text{type} + \text{level} + \text{type}*\text{level} + \text{viscosity}$$

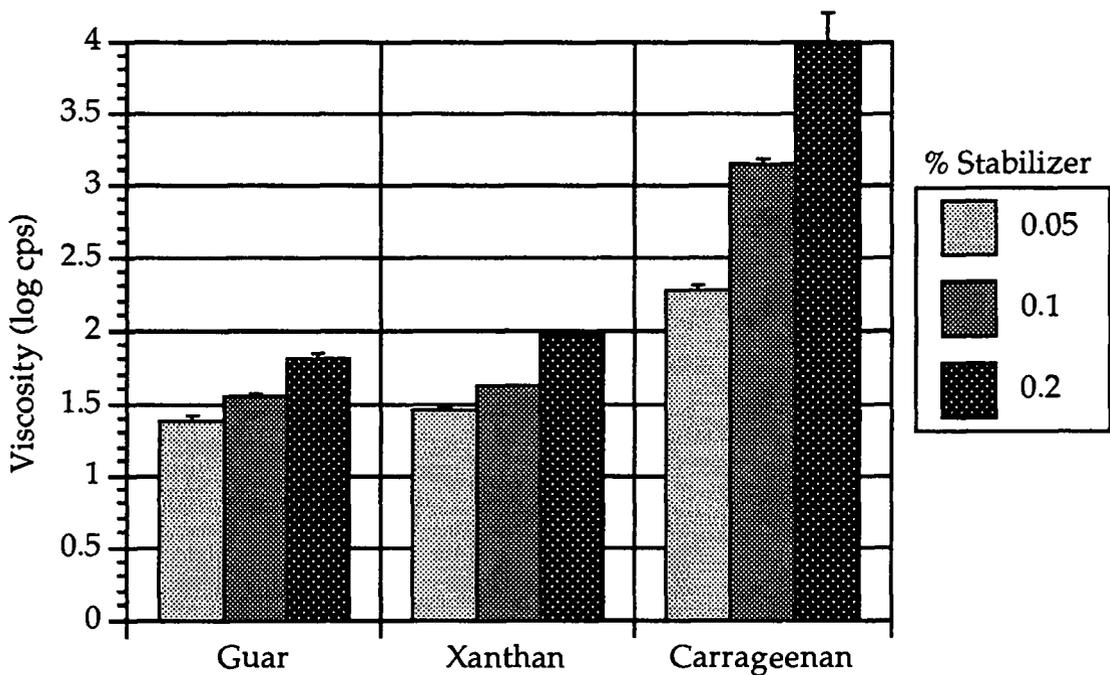
where viscosity was treated as a covariate. Further analysis of viscosity\*type interactions were examined where viscosity was not a class variable:

$$\text{diacetyl} = \text{viscosity} + \text{type} + \text{viscosity}*\text{type}$$

## RESULTS & DISCUSSION

### Stabilizer Effect

Analysis of viscosity data (Fig 4.1) demonstrated diacetyl release inhibition effects due to both stabilizer type and level.



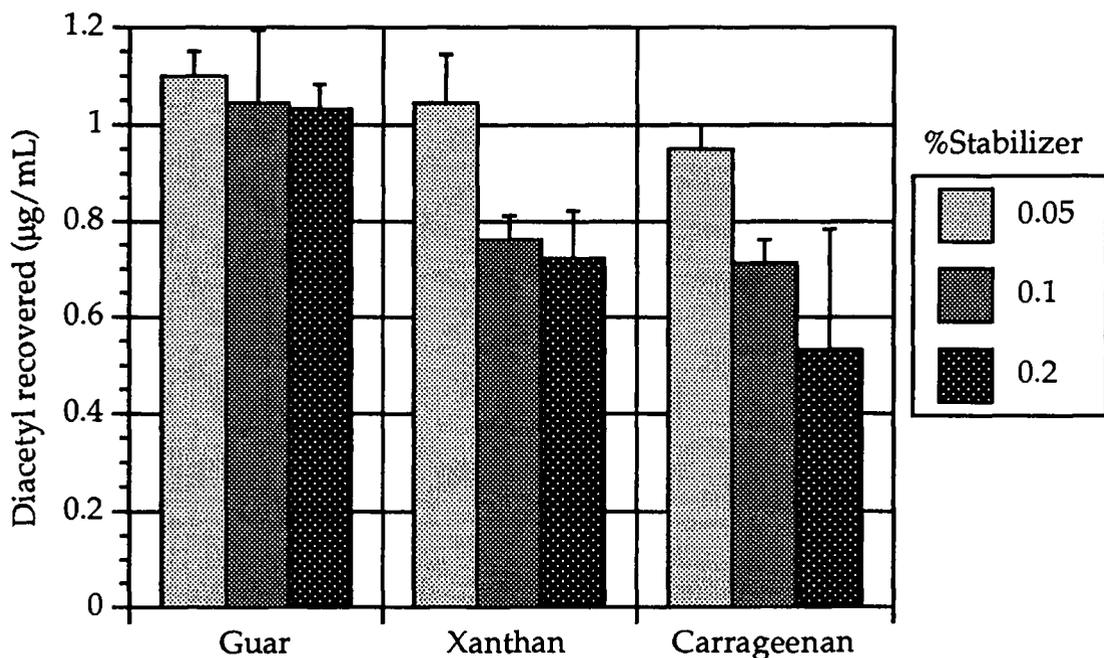
**Figure 4.1.** Log viscosities as a function of stabilizer type and level. Error bars represent one standard deviation about mean values.

High viscosities resulting from carrageenan treatments reflect the previously characterized phenomenon of milk reactivity due to the association of carrageenan with milk proteins (Grindrod and Nickerson, 1968; Schmidt and

Smith, 1992). Milk reactivity has been defined as the ability of a substance to produce higher viscosities in milk than in water and has been demonstrated to result from the interaction of certain additives with milk proteins.

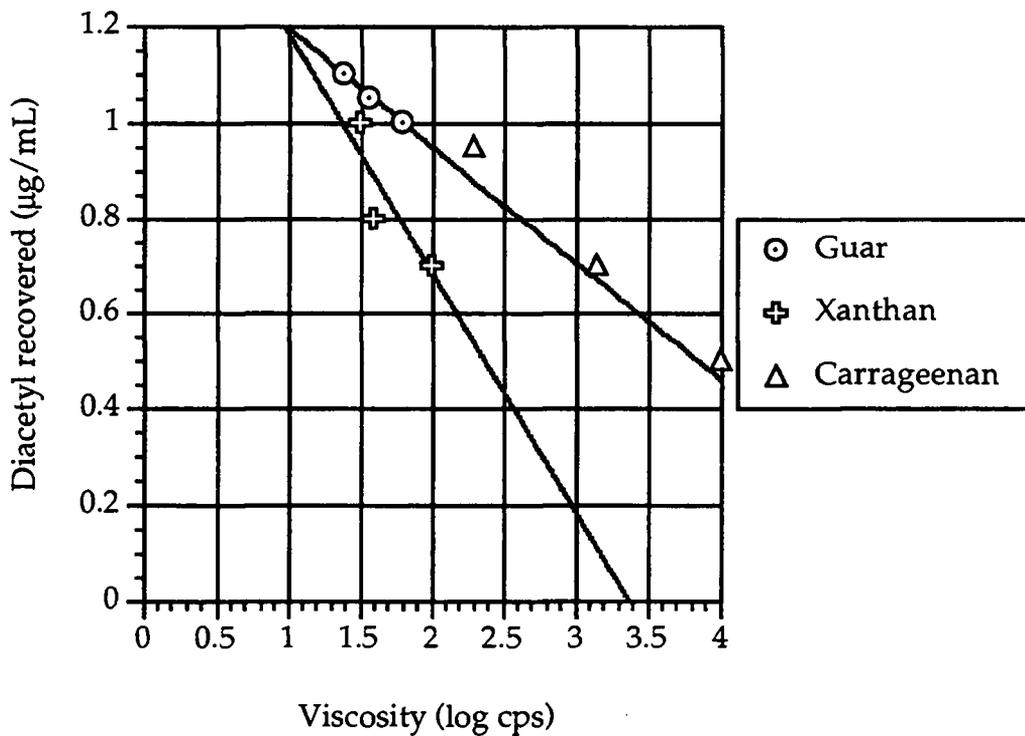
Although guar and xanthan gums have also been shown to exhibit milk reactivity, carrageenan has been shown to be the most reactive and impart the greatest viscosities with a given level of stabilizer (Schmidt and Smith, 1992).

The effect of stabilizer type and level on diacetyl recovery is shown in Fig. 4.2.



**Figure 4.2.** Headspace available diacetyl as a function of stabilizer type and level. Error bars represent one standard deviation about mean values.

These results mirror viscosity results, suggesting that either level or, most likely, viscosity itself inhibits the release of diacetyl from a model system matrix. Statistical analysis demonstrated a significant viscosity\*type interaction ( $P < 0.01$ ) that is displayed in Fig. 4.3.

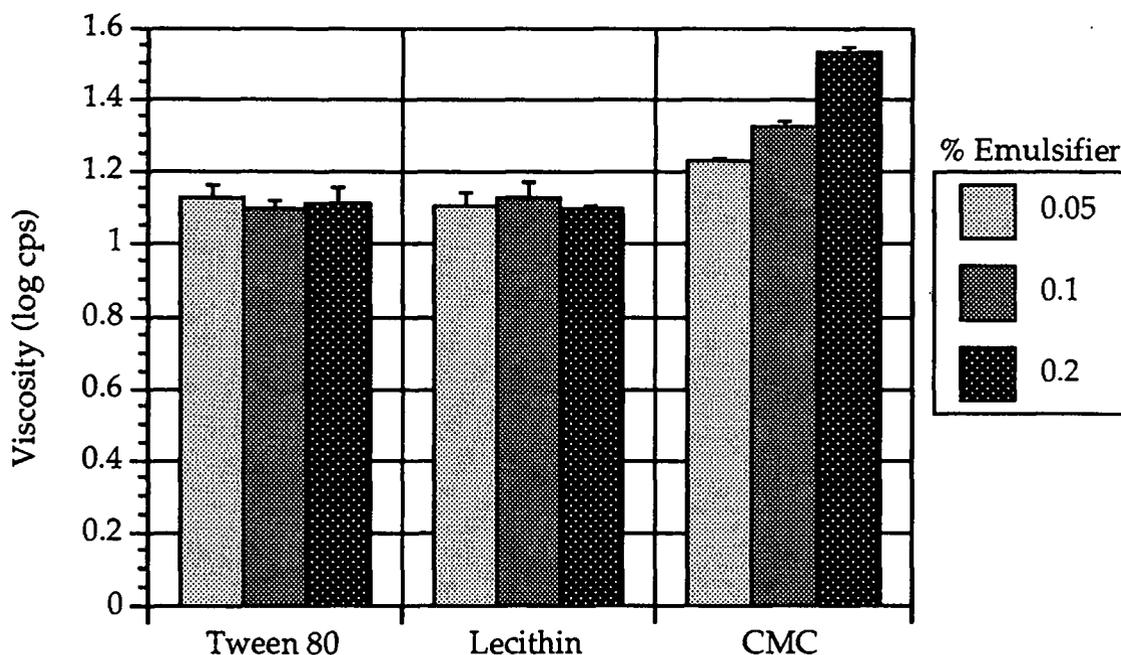


**Figure 4.3.** Effect of viscosity on diacetyl recovery. While guar and carrageenan lie on statistically not different slopes, xanthan gum exhibits increased amounts of diacetyl inhibition for a given increase in viscosity.

While carrageenan and guar gum exhibit a similar rate of diacetyl inhibition when corrected for viscosity, xanthan gum treated samples showed an increased rate of diacetyl inhibition when plotted against viscosity. This suggests that for a given increase in viscosity, xanthan gum will result in the greatest inhibition of diacetyl release. This inhibitory process may result from unique structure and functional moieties on xanthan gum, namely free carboxyl groups. While carrageenan also has sulfate groups, carrageenan's high degree of milk reactivity suggests that these groups may be more closely associated with milk proteins, hence less available for binding interactions with diacetyl. Although diacetyl has no ionizable groups, localized charges associated with the electron withdrawing effect of adjacent carbonyl groups, providing both  $\delta^-$  and  $\delta^+$  regions that may provide the necessary binding sites.

### **Emulsifier Effect**

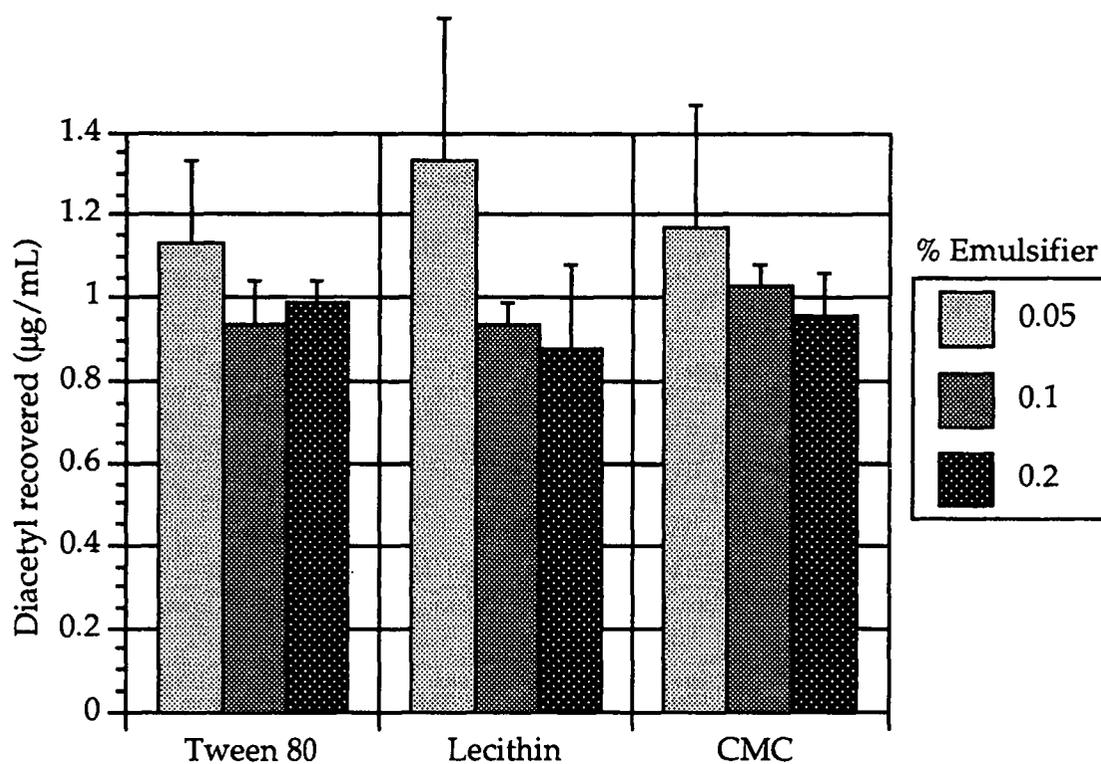
As expected, neither Tween 80 nor lecithin imparted any detectable viscosity as a function of type or level compared to the control. CMC increased viscosity as compared to the control and as a function of level (Fig. 4.4). CMC has been reported to function as both a stabilizer and an emulsifier; imparting viscosity through the formation of a CMC-protein complex (Whistler and Daniel, 1985).



**Figure 4.4.** Log viscosities as a function of emulsifier type and level. Error bars represent one standard deviation about mean values.

Diacetyl release inhibition was greatest for CMC (Fig. 4.5). However, the effect may be due to the resulting viscosity and not from any active binding of diacetyl. Tween 80 exhibited no effect on recoverable diacetyl as compared to the control over the three levels tested. No inhibition was expected since Tween 80 imparted no viscosity and has no charged groups, hence no potential ability to strongly associate with diacetyl. Lecithin did inhibit the release of diacetyl into the headspace as a function of emulsifier level. Although lecithin imparted no detectable viscosity, the effect of

diacetyl release inhibition may be due to the charged nature of the molecule allowing association, and hence, binding of diacetyl. The charged groups on lecithin are unable to associate with milk proteins as demonstrated by the absence of viscosity increase, thus these charged groups are free to associate with diacetyl.



**Figure 4.5.** Headspace available diacetyl as a function of emulsifier type and level. Error bars represent one standard deviation about mean values.

## CONCLUSIONS

Our study suggests that careful choice of type and level of stabilizing agents may be warranted to avoid decreases in headspace available diacetyl. While this study demonstrates the inhibition of a single compound, diacetyl, numerous other aroma-imparting fermentation products, added artificial or natural flavors, or even undesirable flavor-imparting compounds may also be susceptible to binding by stabilizing agents (Puspitasari et al., 1991); Further work may also include sensory examination of the binding phenomenon we have described in this research.

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## CHAPTER 5

### SOLVENT DESORPTION DYNAMIC HEADSPACE ANALYSIS OF CHEDDAR CHEESE\*

Scott A. Rankin and Floyd W. Bodyfelt

\*This chapter was submitted to the Journal of Food Science (Nov., 1995) and is in review.

## ABSTRACT

A rapid dynamic headspace sampling technique was evaluated for its ability to differentiate between Cheddar cheese samples for volatile aroma compounds. Seven commercial brands of Cheddar cheese were examined which ranged in flavor from mild to extra sharp (matured). A total of 14 volatile compounds were tentatively identified by comparison with published retention indices and retention times of known standards. Major volatiles recovered were 2-butanol, acetoin, propanoic acid, butyric acid, and caproic acid. Other identified compounds were 2-butanone, diacetyl, ethyl butyrate, 1-butanol, ethyl caproate, hexanol, acetic acid, 2,3-butanediol, and octanoic acid. Coefficients of variation were relatively small and allowed differentiation of cheese samples examined by triplicate analysis.

## INTRODUCTION

The detection of compounds responsible for the typical aroma of Cheddar cheese has been the focus of numerous research efforts. Over 200 volatile compounds have been identified in Cheddar cheese (Maarse and Visscher, 1989). Although the optimum levels and combinations of compounds responsible for optimal Cheddar flavor have not been conclusively determined, current research suggests that Cheddar aroma is produced by far fewer compounds (Dacremont and Vickers, 1994; Christensen and Reineccius, 1995).

Various methods have been developed for the analysis of aroma compounds found in cheese. Typical methods of analysis include molecular distillation (Christensen and Reineccius, 1995), thermal desorption purge and trap, (Arora et al., 1995), distillation and extraction (Barbieri et al., 1994), dialysis (Vandeweghe and Reineccius, 1990), various types of solvent extraction (Wong and Park, 1968; Anderson and Day, 1966), and direct injection of cheese oil (Liebach, 1970).

Each of these methods has disadvantages that would ultimately limit their application for analysis of aroma compounds on a routine basis. Our recent work with solvent desorption, dynamic headspace sampling suggests that this technique may provide a useful tool for the routine analysis of aroma-imparting compounds in Cheddar cheese. Advantages of our method include rapid sample preparation, inclusion of both lipid and aqueous phase

of cheese, low expense, and quantitative performance (Rankin and Bodyfelt, 1995). The objective of our research was to characterize the effectiveness of a commercially available adsorbent trap to quantitatively analyze Cheddar cheese samples for aroma imparting compounds.

## **MATERIALS & METHODS**

### **Cheese Samples and Preparation**

Cheddar cheeses of varying age and manufacturer were obtained from local producers or purchased locally and stored at 4°C until analysis. Flavor characteristics of samples were informally evaluated by expert cheese judges (Table 5.1). Slurries were prepared for analysis by placing 20.0 g cheese sample and 70 mL boiled, distilled, deionized water into an Osterizer blender at liquification speed for 1 min. The blended slurry was placed into a 150 mL glass bottle with Teflon-lined cap and stored no longer than 60 min at 4°C until analysis.

### **Dynamic Headspace Analysis**

A 10 g aliquot of sample was transferred into a 25-mL round-bottom flask fitted with a universal inlet adapter. Sodium sulfate (5.00 g) was added to reduce condensation of water vapor on the glassware and trap and to increase volatility of recoverable compounds. Samples with added NaCl did not sufficiently inhibit visible condensation.

**Table 5.1-** Descriptions of full-fat Cheddar cheese samples

<u>Cheese sample</u>	<u>Manufacturer description</u>	<u>Flavor scening description</u>
1	Mild.	High acid, slight bitter, slight cheddar aroma, weakly aromatic, very slight dirty flavor.
2	Mild, raw milk, rennetless, organic, aged over 60 days.	Bitter, unclean, weak acid, brothy, acrid, weak Cheddar aroma/flavor.
3	Extra sharp, aged over 3 yrs.	High Cheddar aroma/flavor, unclean, sulfide, bitter, goaty, slight rancid, sulfide.
4	Extra sharp, aged over 5 yrs.	Nutty, high acid, high Cheddar aroma/flavor, very slight bitter, sulfide.
5	Extra sharp, aged over 3 yrs.	Nutty, high acid, moderate Cheddar aroma/flavor, very slight bitter, very slight rancid.
6	New York State Sharp, aged over 6 months.	Moderate acid, slight bitter, lacks Cheddar character, slight dirty, weak sulfide, high salt.
7	Vermont, extra sharp, aged over 14 months.	Moderate acid, moderate Cheddar, bitter, weak sulfide, very slight unclean.

Sample purge was conducted by immersing the flask in a  $37 \pm 1^\circ\text{C}$  circulating water bath which kept the lipid fraction in the liquid state. Purge conditions were as previously described (Rankin and Bodyfelt, 1995). Samples were purged with purified nitrogen gas at 800 mL/min for 15 min; final purge volume was 12 L.

Commercially available Carbopack and activated charcoal traps (Supelco, Bellefonte, PA) with a bed weight of 100 mg were evaluated for adsorbent performance. The activated charcoal provided poor recovery of volatile compounds and was abandoned early in the study. Following headspace sampling, the 50 mg breakthrough section of the trap was placed in a 2 mL vial and examined for analyte breakthrough. The 100 mg section was placed into a separate 2 mL vial with Teflon-lined screw-top cap. A 250  $\mu$ L aliquot of CS<sub>2</sub> solvent was metered into the vial; the vial was capped and allowed to stand at room temperature for 15 min to allow for desorption to occur.

### **Analyte Identification**

Tentative identification of recovered volatiles was obtained by comparison of analyte retention indices (RI) to an available RI database (Libbey, 1991) and by retention times of pure standards (Sigma-Aldrich, St. Louis, MO). RI were calculated using straight-chain hydrocarbons.

### **Gas Chromatography**

Gas chromatography was carried out with a Hewlett Packard (HP, Avondale, PA) 5890 gas chromatograph with flame ionization detector (FID) under the following conditions. Initial temperature was 40°C isothermic for 6 min; increased by 6°C/min to 210°C and maintained for 15 min. A Restek

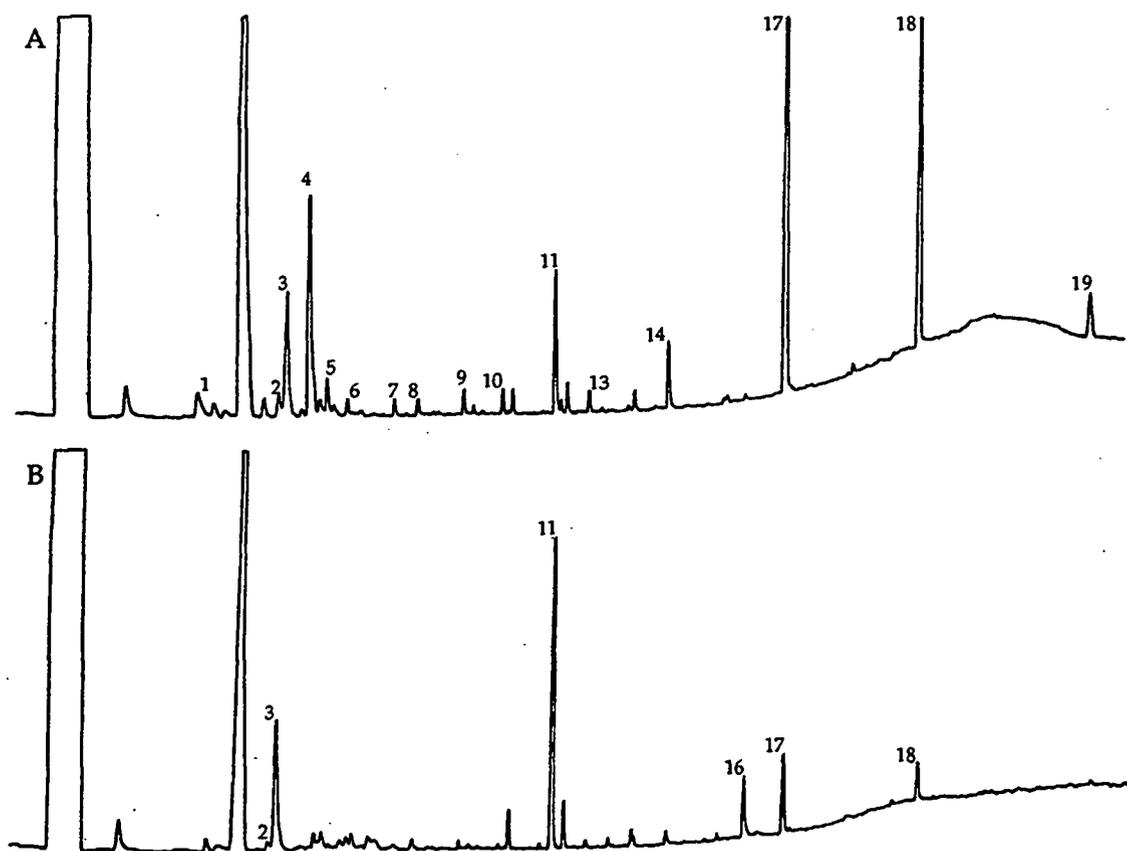
(Bellefonte, PA) Rtx-WAX column was employed (30 m, 0.32 mm i.d., 1.0  $\mu\text{m}$  film thickness). Column flow rate was 1.5 mL/min and sample size was 1.5  $\mu\text{L}$ , splitless injection. An HP 3396 integrator was used for peak area determination. Purge parameters were optimized based on analyte peak areas relative to added external standard, n-decane, which was added to the desorption solvent at a concentration of 10  $\mu\text{g}/\text{mL}$ . Quantitation in actual samples was based on the recovery of 20  $\mu\text{L}$  aqueous added internal standard, methyl butyrate, at a final concentration of 1  $\mu\text{g}/\text{g}$ .

## RESULTS & DISCUSSION

A total of 14 volatiles were tentatively identified in Cheddar cheese samples (Table 5.2). Each of these compounds has been identified in Cheddar cheese samples in previous work. Chromatograms ranged in complexity reflecting the age and flavor characteristics of the samples (Fig. 5.1). The major peaks were found to be 2-butanone, acetoin, butyric acid and caproic acid. While 2-butanone may have no effect on Cheddar aroma (Keen and Walker, 1974), Christensen and Reineccius (1995) suggest that acetoin, butyric acid and caproic acid may be important aroma-imparting compounds in Cheddar cheese. Arora et al. (1995) also suggest that acetoin may be responsible for some part in the character of Cheddar aroma. However,

acetoin was identified in the breakthrough section of the sample traps.

Breakthrough occurred only in samples with high levels of acetoin.



**Figure 5.1.** Typical gas chromatograms from (A) three-year-old Cheddar cheese and (B) mild Cheddar cheese. Peak numbers correlate to numbers in Table 5.2. Peaks without identification numbers are system contaminants.

Table 5.2- Compounds identified in Cheddar cheese samples

No.	RI <sup>a</sup>	Identity	Cheese sample						
			1	2	3	4	5	6	7
1	913	2-butanone	ND <sup>b</sup>	ND	0.59(0.01)	ND	ND	ND	ND
2	995	diacetyl	0.10(0.18) <sup>c</sup>	0.29(0.06)	0.17(0.15)	0.29(0.03)	0.32(0.01)	0.25(0.03)	0.19(0.17)
3	1002	methyl butyrate <sup>d</sup>							
4	1037	2-butanol	ND	5.72(0.86)	3.67(0.39)	5.20(0.35)	ND	ND	ND
5	1055	ethyl butyrate <sup>e</sup>	ND	ND	0.56(0.00)	0.20(0.17)	0.32(0.03)	ND	0.16(0.14)
6	1077		ND	ND	0.21(0.01)	ND	ND	ND	ND
7	1129		ND	ND	0.22(0.01)	1.38(1.87)	ND	ND	ND
8	1156	1-butanol	ND	ND	0.11(0.10)	0.56(0.04)	ND	ND	ND
9	1206		ND	ND	0.28(0.02)	0.04(0.07)	0.32(0.04)	0.14(0.02)	0.18(0.01)
10	1254	ethyl caproate	ND	ND	0.25(0.02)	0.10(0.10)	0.31(0.05)	ND	0.13(0.23)
11	1317	acetoin <sup>e</sup>	2.37(0.21)	3.80(0.02)	1.47(0.11)	ND	2.99(0.22)	2.13(0.12)	1.48(0.15)
12	1328		ND	ND	ND	0.21(0.01)	ND	ND	ND
13	1366	hexanol	ND	ND	0.22(0.00)	0.34(0.02)	0.15(0.03)	0.16(0.01)	0.13(0.11)
14	1470	acetic acid	ND	0.38(0.02)	0.75(0.05)	1.31(0.13)	ND	ND	0.22(0.06)
15	1553	propanoic acid	ND	0.19(0.04)	ND	0.76(0.01)	ND	ND	ND
16	1577	2,3-butanediol	0.46(0.19)	ND	ND	ND	ND	0.64(0.22)	2.64(0.30)
17	1651	butyric acid	0.74(0.24)	1.73(0.22)	10.73(0.28)	5.18(0.50)	14.80(0.29)	1.84(0.16)	2.25(0.08)
18	1865	caproic acid	0.49(0.21)	0.67(0.06)	5.62(0.14)	2.91(0.26)	7.53(0.53)	0.92(0.12)	1.03(0.07)
19	2080	octanoic acid	ND	ND	0.80(0.06)	0.30(0.03)	0.46(0.03)	0.20(0.17)	ND

<sup>a</sup>RI = retention index. <sup>b</sup>ND = not detected. <sup>c</sup>Values in columns represent normalized peak areas followed by standard deviation in parentheses (n = 3). <sup>d</sup>Internal standard. <sup>e</sup>Denotes analyte breakthrough.

Breakthrough may be limited by decreasing purge volume, decreasing sample size, or selecting an adsorbent with a higher capacity for acetoin.

Our method was able to identify diacetyl in all Cheddar samples, albeit at low concentrations. Dacremont and Vickers (1995) suggest that diacetyl is an important component in Cheddar cheese.

Ethyl butyrate and ethyl caproate were identified in four of the seven samples. Although Dacre (1955) concluded that ethyl esters do not contribute to the aroma of typical Cheddar cheese, Bills et al., (1965) reported that ethyl butyrate and ethyl caproate may contribute to the fruity flavor defect.

A total of five free fatty acids were identified in the samples; the highest levels occurred in the most aged cheeses. Although Marsili (1985) demonstrated that aged Cheddar has increased levels of nearly all free fatty acids, Forss and Patton (1966) suggest that butyric and caproic are the only two fatty acids in high enough concentration and with a sensory detection threshold sufficiently low enough to contribute to Cheddar aroma.

## CONCLUSIONS

The ease of operation and low expense of this method may provide a useful analysis method to routinely examine the development of aroma-imparting compounds in Cheddar cheese. Furthermore, the selection of alternate adsorbent materials and adjustment of purge parameters may

potentially allow determination of other volatile compounds believed to contribute to Cheddar aroma.

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## CHAPTER 6. CONCLUSIONS

### **Summary**

The objectives of this study were to examine the performance of a solvent desorption dynamic headspace analysis (SDD) technique applied to the assessment of aroma compounds in fermented dairy products. My results suggest that SDD provides a flexible, low cost approach to objectively test for these compounds.

These results may also provide the background and foundation for future work as techniques are developed for the assessment of other aroma compounds and the assessment of other aroma issues associated with dairy foods.

### **Recommendations for Future Work**

Several areas of research remain open for future investigation. Although we demonstrated the use of a method for diacetyl in buttermilk, this work can be expanded to assay for diacetyl in wine, beer, and other fermented foods where diacetyl is of some significance.

The inhibition of headspace available diacetyl by stabilizers and emulsifiers could easily be expanded to examine the inhibition effect on the release of compounds responsible for other aromas such as vanillin. Work may also investigate the potential of stabilizing and emulsifying agents to

inhibit the release of undesirable compounds such as those responsible for feed flavors.

SDD of Cheddar cheese has the potential to contribute to several areas of importance. First, work should be continued to (1) qualitatively identify volatile compounds having the potential to contribute to Cheddar cheese aroma and (2) determine the concentrations of these compounds in Cheddar cheese. These techniques and tools should ultimately be developed to serve as a tool for the routine assessment of Cheddar cheese aroma compounds. SDD may also be applied to other cheese related issues such as the examination of the flavor chemistry of lowfat Cheddar cheese products.

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