EFFECT OF FAT MIMETICS ON THE HEADSPACE RELEASE OF FIVE STRAWBERRY FLAVOR COMPOUNDS

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by

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The undersigned, appointed by the Dean of the Graduate School,

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A candidate for the degree of Master of Science

And hereby certify, that in their opinion, it is worthy of acceptance.

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ABSTRACT

Consumers prefer to consume low fat foods rather than high fat foods because of increased health consciousness. However, low fat foods have poorer flavor and texture qualities. Fat mimetics are widely used to improve the quality of low fat foods. In this study, the release of five strawberry flavor compounds (*cis*-3-hexen-1-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate, γ-undecalactone, furaneol) from six different emulsion systems (0% fat, 4% fat, 4% fat + Simplesse®, 4% fat + Litesse®, 4% fat + Simplesse®/Litesse®, 10% fat) were determined. The results indicated that the release of *cis*-3-hexen-1-ol, α-ionone, γ-undecalactone and ethyl-3-methyl- 3-phenylglycidate was significantly decreased with increasing fat levels, but furaneol was decreased with decreasing fat levels. Fat mimetics did not affect the release of α-ionone and ethyl-3-methyl-3-phenylglycidate. However, the release of *cis*-3-hexen- 1-ol and γ-undecalactone from 4% fat emulsions was similar to that of a 10% fat emulsion when using Litesse® as fat mimetic, while the release of furaneol from the 4% emulsion was more similar to the release from 10% fat when using Simplesse® as fat mimetic.

CHAPTER 1

INTRODUCTION

Obesity and chronic diseases, such as hypertension and cancer, have become important threats to Americans' health over the last few decades. Most consumers know about the connection between high saturated fat and chronic diseases, so many consumers prefer to consume low fat foods rather than high fat foods. However, consumers want to get the same taste in low fat foods (Koeferli and others 1996). Fat not only plays an important role for the flavor profile, but it acts as a flavor carrier, as well as controls the flavor release from the food matrix (Kinsella 1990). Fat has been reported to influence the qualitative, quantitative and temporal perception of flavor in products (Tuorila and others 1995; Plug and Haring 1994), and low fat foods mostly have poorer flavor quality (Giese 1994; Mela 1995). So, improving the flavor profile of low fat foods has become an important issue in food science. For ice cream, dairy fat plays an important role for flavor release, mouthfeel, appearance, texture and melting profile (Brauss and others 1999). Thus, the main problems of low fat ice cream are poor flavor profile and mouthfeel (Marshall 1991; Hatchwell 1994). Some studies on vanilla and chocolate low fat ice

cream attempted to improve the texture and flavor profile by using fat mimetics (Prindiville and others 2000; Roland and others 1999; Ohmes and others 1998).

In this study, Simplesse® and Litesse® were used as fat mimetics. Simplesse® is a protein-based fat mimetic, and Schirle-Keller and others (1994) reported that Simplesse® behaves like fat and had good interaction with flavors. Litesse® is a carbohydrate-based fat mimetic. Chung and others (2004) reported that there was no significant difference between full fat ice cream and low fat ice cream made with Litesse® in sensory tests. The former research showed that Simplesse® and Litesse® performed very well in improving viscosity and overall texture of ice cream (Schmidt and others 1993). While the effect of fat mimetics on texture is fairly well investigated, their effects on flavor profiles of ice cream are not well researched. This study emphasized how fat mimetics influence flavor release from five strawberry flavor compounds (α-ionone, *cis*-3-hexenol, γ-undecalactone, furaneol and ethyl-3-methyl-3-phenylglycidate).

Strawberry flavor is one of the most popular ice cream flavors in the world. However, research on strawberry flavor ice cream is lacking (Miettinen and others 1999). The main problem of reducing fat in strawberry ice cream is that the flavor would be dramatically influenced by changing the fat content, so using fat mimetics to improve the flavor

profile becomes very important for consumer acceptability of reduced fat strawberry ice cream.

Solid-phase microextraction (SPME) is a sensitive method for volatile compound analysis that has been developed by Pawliszyn and co-workers (Arthur and Pawliszyn 1990). An adsorbent attached to a fused silica fiber is used to extract the volatile compounds from the headspace of the samples. Fused silica fiber coated materials, which have different sorption characteristics, are useful in differentiating between flavor compounds with different chemical characteristics (Rocha and others 2001). Headspace solid-phase microextraction (HS-SPME) has been widely used in food/flavor researches such as for quantification of volatile compounds in beers (Jelen and others 1998) and coffee (Yang and Peppard 1994; Bicchi and others 1997). Combining HS-SPME with gas chromatography not only can efficiently extract flavor compounds but can also quantify flavor compounds very precisely.

The primary objective of this study is to determine how fat mimetics affect the headspace release of five strawberry flavor compounds.

CHAPTER 2

LITERATURE REVIEW

2.1 Strawberry Flavor

2.1.1 Introduction

There are more than 360 flavor compounds that have been found in strawberry (Latrasse 1991). The main flavor compounds in strawberry are aldehydes, alcohols, sulfur compounds, esters and furanone-derived compounds (Schreier 1980; Dirinck and others 1981). Due to the great variety of strawberry flavor compounds, there are many different analysis methods that have been used for the identification or quantification of these compounds. Gas-liquid chromatography (GLC) equipped with a flame ionization detector (FID) is the most popular quantitative analysis method, and GLC equipped with a mass spectrometer is the most powerful identification method for strawberry flavor compounds. However, some strawberry flavor compounds are sensitive to heat, which is the reason for high-performance liquid chromatography (HPLC) also becoming one of the more popular analysis methods for strawberry flavor studies (Zabetakis and Holden 1997). In addition, nuclear magnetic resonance (NMR) (Mayerl and others 1989), gas

chromatography/ mass spectrometry/ fourier transform infra red (GC/MS/FTIR) (Nikiforov and others 1994), HPLC-UV (Sanz and others 1994) and mass spectrometric selected ion monitoring (SIM) (Hirvi 1983) have been applied to strawberry flavor analysis.

Strawberry flavor has not only been extensively studied, but strawberry-flavored ice cream is also the third most popular ice cream after vanilla and chocolate ice cream in the United States (Giese 1994). In this study, five artificial flavor compounds, *cis*-3-hexen-l-ol, γ-undecalactone, α-ionone, ethyl-3-methyl-3-phenylglycidate and furaneol were used to prepare a simple but clearly recognizable strawberry flavor.

2.1.2 *Cis*-3-hexen-1-ol

Cis-3-hexen-1-ol ($C_6H_{12}O$, Mw 100.16) (Figure 2.1) is also called Blätter (German for leaf) alcohol, β,γ-hexenol, *m*-β-hexenol and leaf alcohol. It is an achromatic oily liquid, and can finely dissolve in most common organic solvent and is slightly soluble in water (Burdock 1994). The headspace flavor release of *cis*-3-hexen-1-ol from strawberry ice creams with different fat contents (0, 9 and 18%) was determined by Miettinen and others (2002). The result showed that flavor release of *cis*-3-hexen-1-ol is significantly

influenced by fat content. However, de Roos (1997) showed that *cis*-3-hexen-1-ol is a water-soluble compound, and its volatility in an emulsion system is hardly influenced by increasing fat content. Similarly, de Roos and Wolswinkel (1994) showed that *cis*-3-hexen-1-ol has similar correction factors in water and milk samples. From these evidences, fat can be considered a factor that will influence the release of *cis*-3-hexen-l-ol from emulsions, but the effect on *cis*-3-hexen-1-ol might not be as manifest as it is for other more lipophilic flavor compounds. *Cis*-3-hexen-1-ol can be either extracted from a natural source or manufactured by chemical synthesis. It was found in raspberries (Larsen and Poll 1990), tomato (Buttery and others 1990), mango (Sakho and others 1997), strawberry and guava (Pino and others 2001). *Cis*-3-hexen-1-ol is a very important flavor compound in the overall raspberry flavor profile. The concentration levels of *cis*-3-hexen-1-ol found in ten raspberries varieties ranged from 0.06 to 0.47 mg/kg, and its odor threshold ranges from 0.1 to 1 mg/kg (Larsen and Poll 1990). The difference in the flavor profile between fresh tomato and tomato paste is due to the total loss of *cis*-3-hexen-1-ol from the tomato paste (Buttery and others 1990). Also, *Robinia pseudacacia* and mulberry leaf oil contain more than 50% of *cis*-3-hexen-l-ol, and up to 30% of *cis*-3-hexen-l-ol is found in green tea oil. For the chemical synthesis of

cis-3-hexen-1-ol, sodium acetylide is first ethylated to 1-butyne, and then 1-butyne reacts with ethylene oxide to form 3-hexyn-l-ol. In the presence of palladium, 3-hexyn-1-ol is hydrogenated to yield *cis*-3-hexen-l-ol (Bauer and others 1997).

Cis-3-hexen-1-ol has an intense green odor and has a freshly cut grass or leafy odor after dilution. It is very commonly used in the perfumery and food industries. In the food industry, it is applied to non-alcoholic beverages, ice cream, ices, candy and baked goods (Burdock 1994; Bauer and others 1997).

2.1.3 α-Ionone

Other names for α-ionone include α-cyclocitrylideneacetone and 4-(2,6,6-trimethyl-2-cyclohexen-l-yl)-3-buten-2-one ($C_{13}H_{20}O$, Mw 192.30) (Figure 2.2). It is an achromatic to pale yellow liquid, and the best solubility of α–ionone is 1:3 in 70% alcohol. The proportion retained (%) of α–ionone in aqueous solutions are as follows: 30% ethanol > 1% CMC > 25% sucrose > water (de Ross and Wolswinkel 1994). de Ross (1997) also showed that olive oil strongly decreased the release of α–ionone from the CMC (carboxymethyl-cellulose) emulsion system. Thus, α–ionone is considered a lipophilic flavor compound. It can be either extracted from a natural source or

manufactured by chemical synthesis. In nature, it was found in ten raspberry varieties (concentration levels from 0.72 to 1.81 mg/kg) (Larsen and others 1990) and the essential oils of *Sphaeranthus indicus* L. and *Acacia farnesiana* (Burdock 1994). It is chemically sythesized from pseudoionone, which is made by the condensation of citral with acetone and further cyclization by acid-type reagents (Burdock 1994).

α–Ionone has a sweet floral-woody note and violet-like odor (Brenna and others 2002). It has been applied in the perfumery and the food industry and is an essential material in the synthesis of many relevant products (Brenna and others 2002). In the food industry, it is widely used in non-alcoholic beverages, ice cream, ices, candy, baked goods, gelatins, puddings, chewing gum and icings (Burdock 1994; Bauer and others 1997).

2.1.4 Ethyl-3-methyl-3-phenylglycidate

Ethyl-3-methyl-3-phenyglycidate ($C_{12}H_{14}O_3$, Mw 206.24) is also called aldehyde C-16, ethyl-α,β-epoxy-β-methyl-hydrocinnamate, ethyl-α,β-epoxy-β-methylphenyl propionate, ethyl-2,3-epoxy-3-phenyl-butanoate, ethyl-methyl-phenyl-glycidate, strawberry aldehyde and strawberry pure (Burdock 1994; Bauer and others 1997).

Etyl-3-methyl-3-phenyglycidate is an achromatic to pale-yellow liquid and is generally composed of *cis* and *trans* isomers (Figures 2.3, 2.4). The solubility is poor in water but it has good solubility in most common organic solvents (Burdock 1994). Etyl-3-methyl-3-phenyglycidate is a synthetic flavor chemical that has not been found in the nature. The commercial product is formed by the reaction of acetophenone with the ethyl ester of monochloroaceric acid in an alkaline condensing agent. Depending on the conditions of the reaction, the ratio of *cis* and *trans* isomers in the mixture range from 8:2 to 6:4, and each isomer has a characteristic odor (Bauer and others 1997). In 1977, Mosandl (1977) successfully separated the *cis* isomer from the mixture and the result showed that the *cis* isomer has a more intense and finer flavor than the mixture.

The commercial ethyl-3-methyl-3-phenylglycidate has a strong fruity odor that resembles strawberry and has a slightly acid taste reminiscent of strawberry after dilution. This flavor compound is widely used in perfumery, such as for fruity notes in household products (Bauer and others 1997). In the food industry it is used in various products, such as non-alcoholic beverage, ice cream, ices, candy, baked goods, gelatins, puddings and chewing gum (Burdock 1994).

2.1.5 γ-Undecalactone

γ-Undecalactone ($C_{11}H_{20}O_2$, Mw 184.28) (Figure 2.5) is also known as aldehyde C-14, γ-heptyl butyrolactone, 4-hydroxyundecanoic acid, γ-lactone, peach aldehyde and undecanolide-1,4 (Burdock 1994). It is a colorless to very pale yellow sticky liquid. The solubility of γ-undecalactone in water is poor, but it has good solubility in most common organic solvents (Bauer and others 1997). Also, γ-undecalactone was shown to have high lipophilicity according to the partition coefficient values (Log P_{oct}=3.30; Log P_{cyc}=2.52) in an octanol/water solvent system (Guth and others 2001). It has a strong fruity, peach-like odor, and a peach-like pungent and sweet flavor after dilution. γ–Undecalactone is widely applied in perfumery and the food industry (Bauer and others 1997). In the food industry, it is commonly used in non-alcoholic beverages, ice cream, ices, candy, baked goods, gelatins and puddings (Burdock 1994).

γ-Undecalactone can be either extracted from a natural source or manufactured by chemical synthesis. The chemical characteristics of γ-lactones in fruits, foods and beverages have been studied by using gas-liquid chromatography, gas-liquid chromatography and mass spectrometry, multidimensional gas chromatography, NMR spectral analyses and liquid chromatography (Mosandl and Gunther 1989; Mosandl and

others 1990). It was found in peach, apricot, passion fruit, hydrolyzed soy protein and butter (Salles and others 1991; Burdock 1994). Since the appearance of lactones in dairy products is considered an off-flavor, Siek and others (1970) determined the taste threshold of several lactones. The result showed that the taste threshold of γ-undecalactone in oil (0.93 ppm) is higher than in water (0.025 ppm), which means its release is larger from water systems than from oil systems. It is chemically synthesized by the reactions of undecylenic acid with 70-80% sulfuric acid, octanol-l plus methylacrylate with di-tert-butylperoxide (Burdock 1994; Bauer and others 1997).

2.1.6 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol™)

2,5-Dimethyl-4-hydroxy-3(2H)-furanone ($C_6H_8O_3$, Mw 128.13) (Figure 2.6) is also called strawberry furanone, pineapple furanone, DHF, DMHF and has the trade name Furaneol™. It is a colorless crystal and water-soluble flavor compound (Pyysalo and other 1979; Bauer and others 1997). Furaneol is commonly found in strawberry (Re and others 1973; Larsen and Poll 1992), pineapple (Rodin and others 1965; Flath and Forrey 1970), raspberry (Honkanen and others 1980; Pabst and others 1991), tomato (Buttery 1995), mango (Sakho and others 1997) and beef broth (Tonsbeek and others 1968).

Furaneol is especially high in strawberries during the ripening stage (Perez and others 1996; Roscher and others 1997). It can be chemically synthesized by cyclization of hexane-2,5-diol-3,4-dione with an acidic catalyst or by the reaction of dialkyl oxalate with dialkyl α–methyldiglycolate in the presence of sodium alkoxide and dimethylformanide (Bauer and others 1997).

There are limited reports about the quantitation and analytical protocol of furaneol because of its thermal instability (Flath and Forry 1970; Shu and others 1985) and its sensitivity to pH. In order to overcome the thermal unstability problem, high performance liquid chromatography (HPLC) was used for the qualitative and quantitative determination of furaneol (Lee and Nagy 1987; Sanz and others 1994). Also, Sen and others (1991) introduced a stable isotope dilution assay for the quantitative determination of furaneol. However, Rouseff and others (1998) showed that furaneol is not thermal unstable under normal GC conditions, and it can maintain stable up to 300ºC. The reason why HPLC analysis showed greater values than GC analysis in previous studies was not due to instability in the GC analysis but because of interfering peaks in the HPLC analysis. The compound is incompletely resolved under HPLC conditions, so the interfering peaks were misinterpreted as being part of the furaneol peak. Concerning the

pH value, Hirvi and others (1980) showed that the optimal pH value for furaneol is pH 4, because furaneol is very unstable at pH either above pH 5 or below pH 3.

Furaneol has a caramel, burnt, sweet, pineapple and strawberry odor (Larsen and Poll 1992; Bauer and others 1997). It is extensively used in making food products such as alcoholic and non-alcoholic beverages, ice cream, candy, jams and jellies (Hirvi and others 1980).

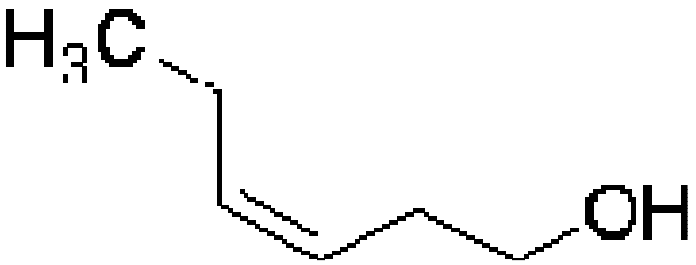

Figure 2.1 Molecular structure of *cis*-3-hexen-1-ol

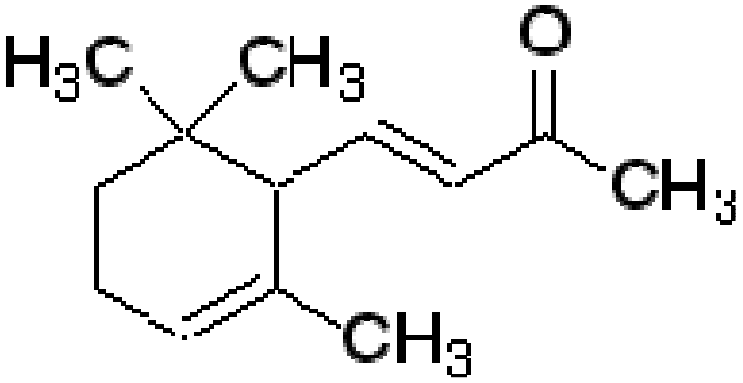

Figure 2.2 Molecular structure of α-ionone

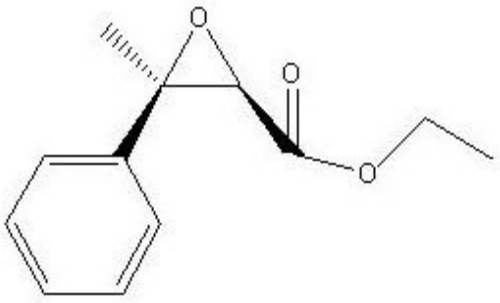

Figure 2.3 Molecular structure of ethyl-3-methyl-3-phenylglycidate *cis* isomer

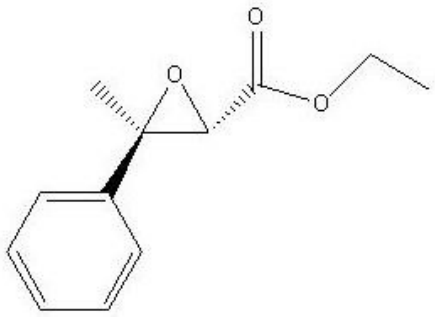

Figure 2.4 Molecular structure of ethyl-3-methyl-3-phenylglycidate *trans* isomer

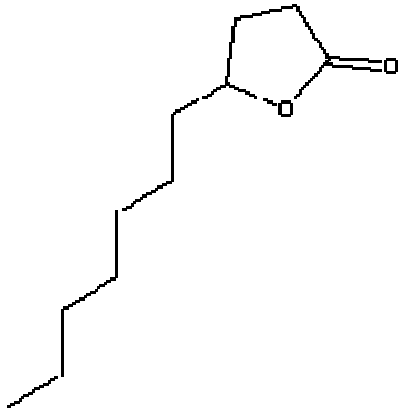

Figure 2.5 Molecular structure of γ-undecalactone

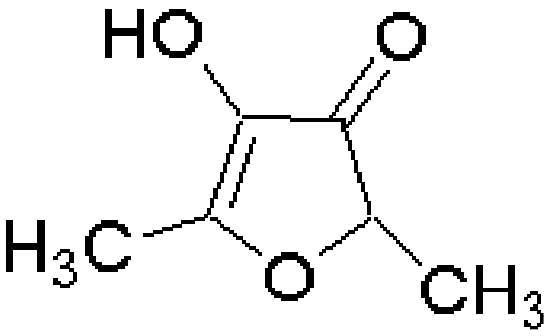

Figure 2.6 Molecular structure of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol)

2.2 Fat

2.2.1 Fat and Flavor

Flavor release from food system is influenced by many food components, such as protein, carbohydrate, polyphenolics, salt and fat. Fat is the most important component because it affects flavor perception, flavor generation and flavor stability (Guichard 2002). Fat dominates the ingredients' effect on flavor perception because it is involved in flavor release, flavor masking, flavor profile and mouthfeel and can even be the source of flavor chemicals, flavor precursor and functions as a flavor reservoir (Forss 1969; Hatchwell 1994). In brief, fat not only plays an important role for the flavor profile, but it acts as a flavor carrier as well as controls the flavor release from the food matrix (Kinsella 1990).

Fat has been reported to influence qualitative, quantitative and temporal perception of flavor in food products (Plug and Haring 1994; Tuorila and others 1995), and low fat foods have been shown to have poor flavor quality (Giese 1994; Mela 1995). For ice cream, dairy fat plays an important role for flavor release, mouthfeel, appearance, texture and melting profile (Brauss and others 1999). Li and others (1997) showed that adding more milk fat in vanilla ice cream would decrease vanilla flavor release. Also, Koeferli

and others (1996) determined that increasing the fat content in vanilla ice cream increases the mouth coating and buttery and creamy notes.

There are some experiments have been done on the strawberry flavor profile with respect to fat contents in food systems including yogurt and ice cream. In a model yogurt system, the strawberry flavor release from low fat content yogurt (0.2%) is faster than that from high fat content yogurt (3.5 and 10%), and the higher fat content decreased correlative particle size and increased viscosity (Brauss and others 1999). In the strawberry ice cream model, Miettinen and others (2002) showed that fat content influences flavor release but the types of fats (vegetable oil or animal fat) does not affect the flavor release. However, Hyvönen and others (2003) found that not only fat content but also the type of fat influences the flavor release from strawberry ice cream. The flavor release from vegetable oil was slightly faster than that from dairy fat. This result was further confirmed by Relkin and others (2004) who showed that different types of fat influenced the flavor release of different flavor compounds. For example, diacetyl was released to a greater extent from the emulsion formed by animal fat, while esters were released to a greater extent from the emulsion formed by vegetable fat.

It is clear from previous studies that flavor profiles and fat levels are obviously

correlated. The lower the fat content the poorer will be the flavor profile. On the other hand, consumers want to purchase low fat food products without sacrificing flavor or taste. To compensate the trade off, fat replacers are commonly used in the production of low fat foods in the food industry.

2.2.2 Fat Replacers

In modern society, people care more and more about their health. Obesity, chronic diseases and cancer have become main health issues for many Americans over the last few decades. Thus, for health reasons, consumers prefer to choose low calorie, low fat foods instead of large portion and high-energy-dense foods. Because of this health consciousness trend, fat replacers have become very common in the food industry because of their functionality of lowering food calories and lowering fat content of foods. Fat replacers not only help calorie control but also provide some of the functionalities that fat would provide in foods. Fat replacers can be categorized as fat substitutes, fat analogs, fat extenders and fat mimetics (ADA Reports 2005). Fat substitutes, which are conventional fats and oils, can replace all the functionalities of fat without contributing any energy (Akoh 1998). Fat analogs, which can replace some functions of fat, are altered

digestibility replacers (Anonymous 2004). Also, fat extenders can reduce the usual amount of fat in food products by improving the functionality of fat (Anonymous 2004). Last but not the least, fat mimetics can replace one or more of the functionalities of fat, but they cannot replace fat on a one-to-one basis (Akoh 1998). In this study, fat mimetics were chosen to replace the fat content in the oil/water emulsion systems.

Fat mimetics can be categorized into two categories: carbohydrate-based and protein-based. There are eight types of carbohydrate-based fat mimetics, such as polydextrose (e.g. Litesse, Gelcarin and Sta-Lite), pectin (e.g. Grindsted and Splendid), maltodextrin (e.g. Paselli and Maltrin), hydrocolloid gums (e.g. Kelgum, Keltrol and Kelcogel), grain-based fiber (e.g. Betatrim and Z-trim), fruit-based fiber (e.g. WonderSlim, dried plum paste, prune paste), cellulose (e.g. Avicel and Just Fiber) and dextrins and modified starches (e.g. Stellar, N-Lite-S and Inscosity). Protein-based fat mimetics can be divided into two types: microparticulate protein (e.g. Simplesse) and modified whey protein concentrate (e.g. Dairy-Lo) (ADA Reports 2005).

Fat mimetics have been commonly used in the many food products including liquid food systems, baked goods, confectionery, dairy products, sausage products, cereal and grain products, fats and oils, prepared entrees, soups, sauces, gravies, snacks

(Anonymous 1992; Yackel and Cox 1992; ADA Reports 2005). In this study, two kinds of fat mimetics, Litesse® (carbohydrate-based) and Simplesse® (protein-based), were used to partially replace the fat in each emulsion system.

2.2.3 Fat Mimetic: Litesse®

Litesse® (polydextrose) which is formed by the polymerization of glucose, citric acid and sorbitol is one kind of carbohydrate-based fat mimetic or sucrose replacer. It can be used as a fat mimetic or as a low-calorie (1 kcal/g) bulking agent, because it has some of the functionalities of fat and it can bind with fat and other fat replacers to become a bulking agent and provide ideal viscosity in low-energy-dense foods (Giese 1996; Kuntz 1996; Alexander 1997). Litesse® is widely used in fat-reduced products, such as ice cream. It works well for the foam stabilization by strong hydrogen bonding and by enhancing film strength in ice cream (Alexander 1997). Because of the polar chemical characteristics, Litesse® is functions poorly for improving the flavor profile in fat-reduced products since most flavor compounds are more lipophilic. Welty and others (2001) confirmed that chocolate ice cream, which contained Litesse®, had a lower concentration of 2-methyl-5-propyl pyrazine than ice creams containing Litesse®.

Litesse® is very heat and cold stable, so it is commonly used to replace fat or sucrose in baked goods, frozen dairy products, puddings, gelatin, candy, salad dressing, syrups, fruit spreads and chewing gum (Giese 1996; Akoh 1998). Also, Litesse® has many other benefits such as being low caloric, fiber-enhanced, reducing glycemic load, tooth friendly, being a prebiotic and improving digestive health (Anonymous 2007[b]). However, it has a laxative side effect. Products containing more than 15 grams per serving need to be clearly labeled regarding the side effects (Akoh 1998).

2.2.4 Fat Mimetic: Simplesse®

Simplesse® is a protein-based fat mimetic (microparticulate protein) and made from whey protein. It is both available in gel form and powder form (Anonymous 2004; Alexander 2005). Microparticulate proteins are round particles with 0.5 to 2.0 micrometers in diameter, and when hydrated in aqueous solution they give the same mouth feel as fats (Kuntz 1996; ADA Reports 2005). They provide 4 kcal/g on a dry basis which is half of the calories provided by fat (Akoh 1998). Simplesse® is often used as fat mimetic in food products, and it provides several benefits, such as fat reduction, fat-like mouth feel, water-binding capacity, slow melt-down rate and gel formation (ADA

Reports 2005). Simplesse® was shown to have better interaction with flavor compounds, especially with aldehydes, than carbohydrate-based fat mimetics (Schirle-Keller and others 1992, 1994). However, Simplesse® still could not totally replace fat in the flavor profile. Welty and others (2001) showed that flavor release is increase when the fat is replaced by fat mimetics. For example, the flavor release of 3,5-diethyl-2-methyl and 2-methyl-5-propyl pyrazines is lower from chocolate ice cream containing milk fat than that of ice cream containing cocoa butter or fat mimetics.

Simplesse® is not suitable for fried food products, but it is good for making ice cream, frozen desserts, salad dressing, margarine, mayonnaise, butter, sour cream, cheese, yogurt, baked goods, soups, dips, frostings and sauces (Akoh 1998; ADA Reports 2005). Since Simplesse® is manufactured from whey protein; it still has all the biological characteristics of protein. Thus, consumers who are allergic to protein should not consume products made with Simplesse® (Akoh 1998).

2.3 Gas Chromatography

2.3.1 History and Principle of GC

Gas chromatography is one of the most common analytical separation methods. It can provide fast and high resolution separations of a variety of compounds, especially volatile compounds. The idea of gas chromatography was developed by Martin in 1944, and the first gas chromatography publication was published in the Biochemical Journal by James and Martin in 1952 (Bartle 1993). Three years later, the first gas chromatography instrument was available on the market. After being commercialized, innovations, such as open tubular column, flame ionization detector, electron capture detector, fused silica capillary column, GC-mass spectrometry and GC-fourier transform infra red were developed one after the other (Grant 1996). These developments allowed gas chromatography to become the most powerful and the easiest analytical separation method for a wide range of compounds.

A sample is analyzed in a GC by the following procedure: First, a small amount of sample is injected into the injector port. Second, the sample is fully vaporized in the injector port and delivered into the chromatographic column. To perform the analysis, a specific flow rate of carrier gas carries the analytes through the chromatographic column

while the column is held in an oven. Depending on the different chemical characteristics of each chemical, the compounds are well separated by the mobile phase (carrier gas) and the stationary phase (column) in the chromatographic column. The detector is connected at the end of the column to monitor the separated compounds, and it will give electrical signals to the recorder. The recorder will further transform the signals to meaningful data, i.e. a chromatogram, via a computer program.

2.3.2 GC Instruments

A gas chromatograph (Figure 2.7) is basically equipped with a tank holding the carrier gas, a flow controller, an inlet system (injector port), a chromatographic column, an oven, a detector and a recorder (Tipler 1993). Depending on different analytical purposes and different chromatographic columns, different carrier gases (mobile phase) such as helium, nitrogen, hydrogen, air, oxygen, argon, argon/methane and helium/hydrogen are commonly used in gas chromatography (Tipler 1993). To obtain the best result, the proper amount of sample needs to be injected quickly into the inlet system, so the inlet system is very critical. According to a sample's conditions and different analytical purposes, split injection, splitless injection, direct injection, programmed-temperature injection,

headspace analysis and solid phase microextraction injection are widely used (Yuwono and Indrayanto 2005). The chromatographic column is the core of the gas chromatograph. There are two types of columns: packed and capillary (open tubular) column. Packed columns have lower theoretical plate numbers and thus, lower resolution than capillary columns, but they have better capacity for large quantities of sample. However, capillary columns have higher efficiencies, better separation ability, better inertness, longer life, are more suitable for use with spectroscopic detectors and have lower column bleed (Yuwono and Indrayanto 2005), which are the reasons why capillary columns are more commonly used. Numerous stationary phases, such as 100% dimethyl polysiloxane, 5% phenyl 95% dimethyl polysiloxane, 14% cyanopropylphenyl methyl polysiloxane and polyethylene glycol are available for capillary GC analysis, and the choice of stationary phase depends on the analytical compounds of interest (Reinceccius 2003). The oven also plays an important role in GC analysis as it controls the temperature of the column. There are four types of ovens available on the market including a conventional GC oven, a flash GC oven, a microwave GC oven and an infrared heated GC oven (Yuwono and Indrayanto 2005). Many different detectors are available, such as flame ionization detector, thermal conductivity detector, electron capture detector, nitrogen-phosphorus

detector, flame photometric detector, atomic emission detector and mass spectrometer (Reinceccius 2003); each detector has its specific analytic sensitivity or selectivity.

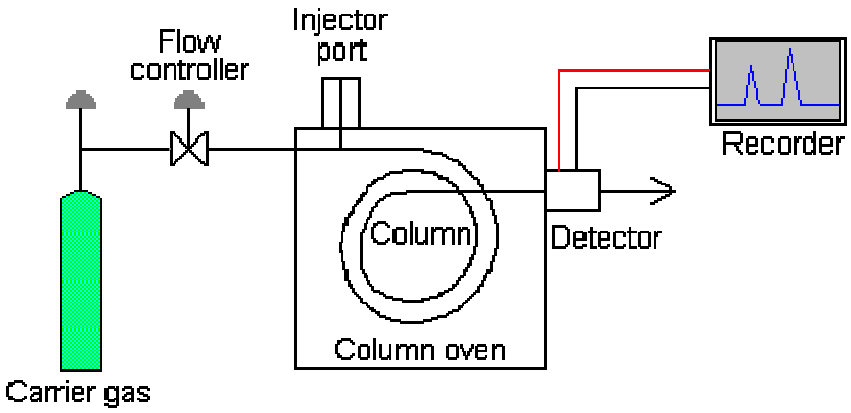

Figure 2.7 The basic components of a gas chromatograph (Anonymous 2007[a])

2.3.3 Application and Recent Developments in GC

Gas chromatography has been a common analytical tool in many research areas, such as toxicology, chemistry, medical science, environmental analysis, petroleum exploration, biotechnology, and food science for decades. In the food science area, GC is widely used for the analysis of flavor compounds, additives, pesticides, drugs, lipids, simple sugars, amino acids, vitamins, antioxidants, and many more (Reinceccius 2003). For the analysis of the strawberry flavor profile, a gas chromatograph equipped with a flame ionization detector is used because of its high sensitivity towards variety organic compounds

(Pyysalo and others 1979; Dirinck and others 1981; Pickenhagen and others 1981). For the identification of the strawberry flavor compounds, gas chromatography connected to a mass spectrometer is an effective tool (Pyysalo and others 1979; Dirinck and other 1981; Perez and others 1992). Also, gas chromatography was used to determine the changes in the strawberry flavor profile during freezing (Douillard and Guichard 1990).

There are some new developments in gas chromatography, such as high speed gas chromatography, on-line gas chromatography and portable gas chromatography. High speed gas chromatography does not only increase the selectivity and minimizes the analysis time, but also allows the use of a shorter column, increased flow rate and faster temperature programming (Donato and others 2007). It has been applied for the analysis of citrus oil (Mondello and others 2003), bergamot oil (Bicchi and others 2005), pesticides in vegetables (Arrebola and others 2003), and many more. On-line gas chromatography is very good for the analysis of complex compounds by improving detector restrictions, reducing the analysis time and giving greater reproducibility. It has been used to identify the complex impurities in pharmaceutical products by Ogorka and others (1992). The last one, portable gas chromatography, allows GC analysis outside the laboratory. It is very convenient for environmental and forensic sciences (Yuwono and

Indrayanto 2005).

2.4 Solid-Phase Microextraction (SPME)

2.4.1 Principle of SPME

Solid-phase microextraction is a sensitive method for the analysis of volatile compound that has been developed by Pawliszyn and co-workers (Arthur and Pawliszyn 1990). An adsorbent attached to a fused silica fiber is used to extract the volatile compounds from the headspace of the samples. A fused silica fiber is coated with materials, which have different sorption characteristics that are useful in differentiating between flavor compounds that differ in their chemical characteristics (Rocha and others 2001). SPME is suitable for analyzing non-polar or semi-polar compounds in either gas, liquid or solid phase (Vas and Vekey 2004).

There are two steps in the SPME extraction: First, samples partition onto the fiber coating. Second, the concentrated samples desorb into the analytical instrument (Ibanez and Cifuentes 2001). Basically, the SPME extraction is completed when the concentration of the analyte reaches equilibrium between the sample and the fiber coating. The equilibrium situation between sample and fiber can be presented as:

$$n = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s} \qquad \{equ\ 2.1\}$$

The n is representative of the amount extracted by the fiber coating, K_{fs} is the constant of fiber coating/sample distribution, V is the volume of fiber coating, V_s is the volume of sample and C_0 is the original analyte concentration in the sample matrix. In order to get reproducible data, the equilibrium conditions, such as extraction temperature and time need to be consistent (Pawliszyn 1997).

2.4.2 SPME Apparatus

The solid-phase microextraction apparatus is shown in Figure 2.8. SPME is a syringe-like device, which includes a fiber coated with stationary phase. Depending on the polarity of the stationary phase, the compounds of interest will be selectively extracted onto the stationary phase. There are many different SPME stationary phases commercially available, including polydimethylosiloxane (PDMS), polydimethyl-siloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA), carboxen/polydimethyl-siloxane (CAR/PDMS), carbowax/divinylbenzene (CW/DVB), and many more (Wardencki 2004). Using SPME as an extraction tool has lots of advantages, such as

being fast, durable, organic solvent free, cheap, a simple apparatus, requiring only a small sample size and being very sensitive to particular compounds (Wardencki 2004). On the other hand, there are some limitations when using SPME as an extraction tool, such as the linear range, the extraction time and the competition effect between different compounds and fiber coating (Roberts and others 2000). However, these limitations can be solved by doing some pretest. The optimal linear range and extraction time can be determined in a pretest, and the suitable fiber coating can be chosen after comparing different fiber coatings. Overall, using SPME as an extraction method has many advantages, so, it is widely used in pharmaceutical, environmental, food and flavor, forensic and toxicology analysis (Wercinski and Pawliszyn 1999).

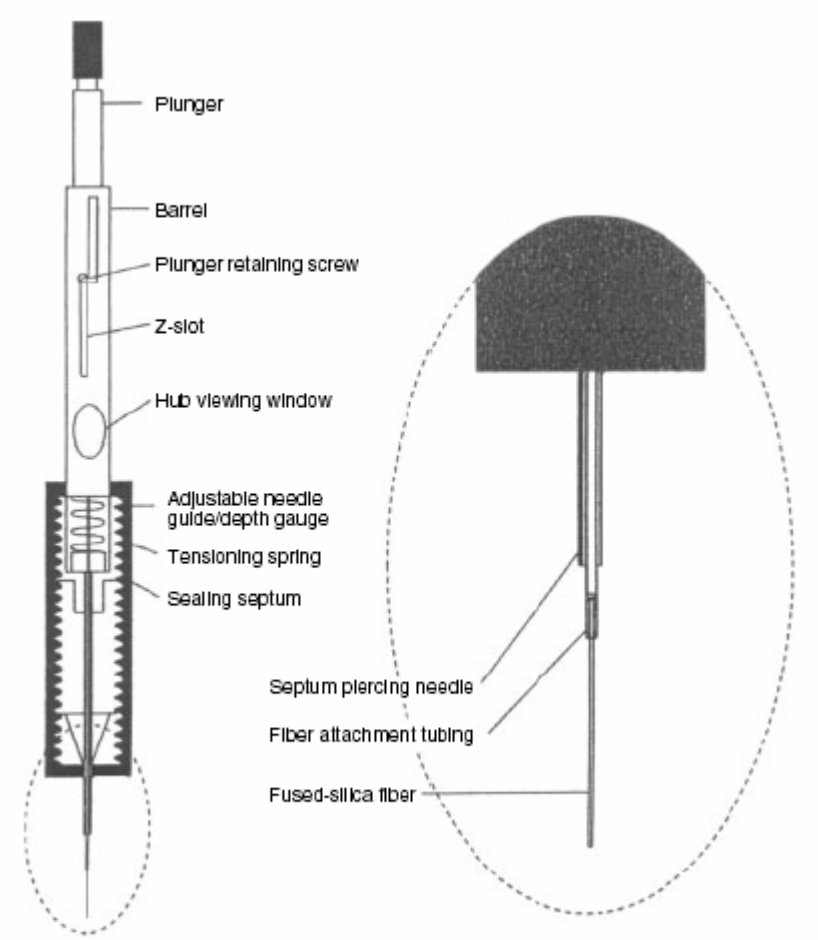

Figure 2.8 The SPME apparatus (Vas and Vekey 2004)

2.4.3 Application of SPME in Food Analysis

SPME extraction has been commonly used in food and flavor analysis for over a decade. Most of the flavor compounds (sulfur compounds, terpenes, lipid-derived aldehydes, etc.) are more non-polar compared with the food matrix (protein, carbohydrate, water, etc.), so the relatively non-polar fiber coating is very suitable for analyzing food

samples (Yang and Peppard 1999). It has been used to analyze aroma compounds in fruits, meats, herbs, alcoholic beverages, coffee, oils, dairy products, seafood, bakery products and tobacco (Wardencki and others 2004). For example, SPME has been used to measure the changes of banana flavor during the freeze drying process. The result showed that the banana flavor compounds are lost completely after five to eight hours of freeze drying (Picque and others 1995). In another example, twenty-nine apple flavor compounds were identified by using SPME (Song and others 1997). Menthol and menthon in chewing gum, volatile aroma compounds in cooked pork, sulphur compounds in beer were successfully measured by using PDMS, CAR/ PDMS and DVB/ CAR/ PDMS SPME fibers (Ligor and Buszewski 1999; Elmore and others 2000; Hill and Smith 2002). SPME has also been used to analyze pesticides, vitamins and antioxidants in food products (Wardencki and others 2004). These research examples show that SPME is a powerful and widely used extraction method in food science.

2.4.4 Quantification Analysis by Using SPME

Using SPME for quantification is somewhat problematic because of competition between compounds, extraction time, linear range and varied affinity to the fiber coating

(Roberts and others 2000). Standard addition to the sample matrix has been shown to not be a good method. It can only be used if standards for all compounds are added, which makes quantification of a complex matrix impossible (Murray 2001). However, the limitation can be rectified by using an external standard curve (Langenfeldt and others 1996). Isotope dilution assay is also a good method to solve quantitation limitations by labeling compounds with isotopes, which then work as internal standards (Roberts and others 2000). After these limitations were, SPME has become a good and easy method to quantify the sample's components.

SPME has been successfully used to quantify flavors in foods, such as beers (Jelen and others 1998) and coffee (Yang and Peppard 1994; Bicchi and others 1997). The caffeine concentration levels in beverage were also quantified by using SPME with isotope dilution assay (Hawthorne and others 1992). In conclusion, combining solid-phase microextraction with gas chromatography not only can efficiently extract flavor compounds but can also be used to quantify flavor compounds very precisely.

2.5 Partition Coefficient

Flavor release from the food matrix is controlled by many factors, such as fat, protein, and carbohydrate content as well as structure. In this study, the release of five strawberry flavors from emulsion systems, which were made from oil and water, was investigated. Fat has already been considered to influence flavor release from emulsion systems. This is because most of the volatile compounds are hydrophobic compounds. The higher the fat content in the emulsion systems, the fewer flavors are released from the system. Guyot and others (1996) showed that hydrophilic compounds (butyric acid and diacetyl) display greater release when the fat content is high. On the other hand, hydrophobic compounds (δ-decalactone) exhibit greater flavor release when the fat content is low. Roberts and others (2003) used nine different emulsion systems ranging from 0 to 4.5 % fat. The results revealed that the hydrophilic compounds showed greater flavor release from emulsions high in fat content, and the flavor releases systematically decreased with decreasing fat content. The hydrophobic compounds showed results opposite to those for hydrophilic compounds.

When people consume food products, flavors can be detected by being released into the headspace of the mouth. The headspace flavors are further transported from the

mouth to the olfactory system, resulting in the human body to perceive the sense of smell. When people have olfaction, partitioning between air and water happens in the mouth. The partition coefficients can be described as follows after reaching equilibrium:

$$P_{aw} = C_a / C_w \quad \{equ\ 2.2\}$$

Here C_a is the concentration of flavor compound in air (g/L), and C_w is the concentration of flavor compound in water. However, most food products also contain fat. The partition coefficient between air, water and fat can be described follows after reaching equilibrium:

$$P_{fw} = C_f / C_w \quad \{equ\ 2.3\}$$

C_f is the concentration of flavor compound in fat (g/L), and C_w is the concentration of flavor compound in water. The flavor compound will be considered more hydrophobic when it has higher P_{fw} value comparing to the other flavor compound (de Roos 1997).

The partition coefficient of emulsion system has been well established by Harrison and others (1997). When the headspace flavor release reaches equilibrium, the relationship between air and emulsion can be written as following:

$$K_{ae} = C_a / C_e \quad \{equ\ 2.4\}$$

Here, K_{ae} is the partition coefficient between air and emulsion. C_a is the concentration of volatile compound in the air, and C_e is the concentration of volatile compound in the

emulsion. To obey the mass balance, the concentration of volatile compounds in the emulsion (C_e) and air (C_a) can be further described as:

$$C_e = (1 - \Phi_d)C_c + \Phi_d C_d \qquad \{equ\ 2.5\}$$

$$C_a = K_{ae}C_c[(1-\Phi_d) + \Phi_d K_{dc}] \qquad \{equ\ 2.6\}$$

C_c is the concentration of volatile compound in the continuous phase, C_d is the concentration of volatile compound in the dispersed phase and dispersed phase's volume fraction is Φ_d. The equilibrium partition coefficient of volatile compound in the headspace can be determined by joining equation 2.4, equation 2.5 and equation 2.6:

$$K_{ae} = \frac{K_{ac}}{1 + (K_{dc} - 1)\Phi_d} \qquad \{equ\ 2.7\}$$

This equation can be used for calculating the partition coefficient either in oil-in-water or water-in-oil emulsion systems.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Flavor Compounds

The following five flavor compounds were purchased from Aldrich Chemical Co, Inc. (Milwaukee, WI).

- *Cis*-3-hexen-l-ol: Purity 98+% (Cat# W256307).
- α-Ionone: Purity 90% (Cat# W259403).
- Ethyl-3-methyl-3-phenylglycidate: Purity 98+% (Cat# W24406).
- γ-Undecalactone: Purity 98+% (Cat# W309109).
- 2, 5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol): Purity 98+% (Cat# W317403).

3.1.2 Emulsion Systems

Different emulsion systems were respectively made from the following ingredients:

- Distilled water
- Great Value unsalted butter (82.14 % fat) (Wal-Mart, Columbia, MO)

- Simplesse®100 (CP Kelco, San Diego, CA)
- Litesse® (Danisco, St. Louis, MO)
- Emulsifier #19 (Opta Food Ingredient Inc., Bedford, MA)

3.2 Instrumentation

3.2.1 Gas Chromatography

A Varian Star 3400CX gas chromatograph with a Varian 8200CX autosampler (Varian Chromatography System Inc., Walnut Creek, CA) was used in this study. The Varian Star 3400CX was equipped with a Model 1078 split/splitless injector and a flame-ionizing detector. Two different kinds of column, a DB-5 and a DB-Wax (Table 3.1), were used in this study. The DB-5 column was used for the analysis of *cis*-3-hexen-l-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate and γ-undecalactone, while the DB-Wax was used for the analysis of 2,5-dimethyl-4-hydroxy- 3(2H)-furanone (Furaneol).

Table 3.1 Detail information of DB-5 and DB-Wax column (J & W Scientific, Folsom, CA)

	DB-5	DB-Wax
Length (meter)	30	30
Film (μm)	0.25	0.25
I.D. (mm)	0.32	0.25
Temperature (°C)	-60 ~ 325	20 ~ 250
Serial number	5900374	7391285

3.2.2 Solid-Phase Microextraction (SPME)

The SPME fibers, 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/ Carboxen™/ PDMS Stableflex) (Cat# 57329-U), were purchased from Supelco (Bellefonte, PA) and used for the flavor analysis. This SPME fiber coating which has bipolar chemical characteristic is suitable for analyzing either polar compounds (furaneol) or non-polar compounds (*cis*-3-hexen-1-ol, α-ionone, γ- undecalactone ethyl-3-methyl-3-phenylglycidate) in this research. The fibers were conditioned at 250 ℃ for an hour before running experiments each day.

3.2.3 Silanizing Sample Vials

Headspace sample vials (10 mL; Cat# 33105-U), open center aluminum seals (Cat# 27230U), teflon coated PTFE/silicones 20 mm septa (Cat# 27514) and a hand crimper (Cat# 33195) were purchased from Supelco (Bellefonte, PA). The septa needed to be conditioned at 150 ºC overnight and the sample vial needed to be silanized before using. All sample vials were silanized with 5% dimethyldichlorosilane (DMDCS) in Toluene (Sylon CT) (Lot# LB06711, Supelco, Bellefonte, PA) to get more accurate results. Silanizing the sample vials will prevent the flavor compounds from attaching to the sample vials (Bartelt 1997). Sample vials were soaked subsequently in two separate Sylon CT solutions each for 5 to 10 seconds. Sample vials were then washed with methanol (Cat# A454-4, Fisher Scientific, Fair Lawn, NJ) and dried with nitrogen (Pre-purified, Lot#401111716274, Airgas Inc., Radnor, PA) at the end.

3.3 Determination of External Standard Calibration Curve

3.3.1 Preparation of Flavor Solutions

The following stock solutions were prepared: 5000 ppm (590 µL/100 mL) of *cis*-3-hexen-l-ol, 100000 ppm (2700 µL /25mL) of α-ionone, 100000 ppm (9200

μL/100 mL) of ethyl-3-methyl-3- phenylglycidate, 10000 ppm (1060 μL/100 mL) γ-undecalactone and 1000 ppm (100 mg/100 mL) of furaneol. Five concentration levels of *cis*-3-hexen-l-ol, ten concentration levels of α-ionone, nine concentration levels of ethyl-3-methyl-3-phenylglycidate, nine concentration levels of γ-undecalactone and five concentration levels of furaneol were further diluted from each stock solution using pentane (Serial# P399-4, Fisher Scientific, Fair Lawn, NJ).

While for cis-3-hexen-l-ol and furaneol one individual external standard curve was used, for α-ionone, ethyl-3-methyl-3-phenylglycidate and γ-undecalactone two external standard curves (low concentration level and high concentration level) were used. The reason for using two separate standard curves for α-ionone, ethyl-3-methyl-3-phenylglycidate and γ-undecalactone was because of the large difference in flavor release from 0% fat emulsion systems compared to the 4% systems. The original standard curve that covered the entire concentration range was insufficiently linear and skewed specifically at the low concentrations. Thus, to get more accurate quantification results, the external standard curves were split into two. Low concentration levels of external standard curves were used for the calculation of 10% fat, 4% fat, 4% fat + Simplesse®, 4% fat + Litesse® and 4% fat + Simplesse®/Litesse®; while high

concentration levels of the external standard curves were used for the calculation of flavor release from 0% fat.

3.3.2 External Standard Calibration Curves

One μL of each diluted solution was directly injected into the GC using a 10 μL Hamilton syringe (Reno, NA). The volatile compound was desorbed at 250 ºC for 6 minutes in the injector port (split mode 1:100), and the FID detector was kept at 275 ºC. Air (Lot# EN00T089A, Airgas Inc., Radnor, PA), hydrogen (Lot# UN1049, Praxair, Danbury, CT) and ultra high purity helium (Lot# 40-1111125538, Airgas Inc., Radnor, PA) were used for the FID detector and as mobile phase, respectively, in the GC. *Cis*-3-hexen-l-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate and γ-undecalactone were analyzed on the DB-5 column using the following temperature program: Initial temperature was 35ºC for 5 minutes, which was increased to 220ºC at the rate of 8ºC/min, and then increased to 250ºC at the rate of 3ºC/min. One run took 38 minutes at a pressure of 8 psi with the flow rate of 1 mL/min. Furaneol was analyzed on the DB-Wax column using the following temperature program: Initial temperature was 50ºC for 5 minutes, which was increased to 250ºC at the rate of 18ºC/min and held for 1.89 minutes. One run

took 18 minutes at a pressure of 20 psi with the flow rate of 1 mL/min. The area count of each flavor compound was integrated by the Star Chromatography Workstation Software (Varian version 4.51, Varian Assoc. Inc., Walnut Creek, CA).

The concentration levels and the actual amounts injected into the GC of each flavor compound are shown in Table 3.2. The external standard calibration curves were drawn using amounts (ng) injected into GC as the independent variable (X-axis) and peak area counts as the dependent variable (Y-axis) (Figures 3.1- 3.6).

Table 3.2 The concentration levels (ppm) and the actual amounts (ng) injected into the GC for preparing the external standard curves.

Cis-3-hexen-1-o1		α-Ionone		γ-Undecalactone		Ethyl-3-methyl-3 -phenylglycidate		Furaneol	
Conc.	Amount	Conc.	Amount	Conc.	Amount	Conc.	Amount	Conc.	Amount
500	5	10	0.1	0.5	0.005	10	0.1	1	0.01
1000	10	50	0.5	1	0.01	50	0.5	10	0.1
3000	30	100	1	10	0.1	100	1	50	0.5
4000	40	250	2.5	50	0.5	500	5	100	1
5000	50	500	5	100	1	1000	10	250	2.5
---	---	1000	10	200	2	2000	20	---	---
---	---	3000	30	500	5	5000	50	---	---
---	---	10000	100	1000	10	10000	100	---	---
---	---	20000	200	2000	20	20000	200	---	---
---	---	30000	300	---	---	---	---	---	---

3.4 Determination of Partition Coefficient

3.4.1 Preparation of Flavor Solutions

Five 1000 ppm stock solutions were made as following: 118 μL (100 mg) *cis*-3-hexen-l-ol, 108 μL (100 mg) α-ionone, 106 μL (100 mg) γ-undecalactone, 92 μL (100 mg) ethyl-3-methyl-3-phenylglycidate and 100 mg furaneol were pipetted or weighed individually into 100 mL volumetric flasks and volumed with pentane (Serial# P399-4, Fisher Scientific, Fair Lawn, NJ). These stock solutions (1000 ppm) were further transferred into screw cap test tubes and kept in the refrigerator. The stock solutions (1000 ppm) were warmed up to room temperature before making dilutions. Depending on the analytical target range, each stock solution was further diluted into three or six concentration levels (Table 3.3). *Cis*-3-hexen-l-ol and furaneol individually got one partition coefficient curve, but α-ionone, ethyl-3-methyl-3-phenylglycidate and γ-undecalactone independently got two partition coefficient curves (low & high conc.). The reason is the same as external standard curve described in 3.3.1.

Table 3.3 The concentration levels (ppm) of five flavor compounds used for the determination of partition coefficient.

Cis-3-hexen-1-o1	α-Ionone	Ethyl-3-methyl-3-phenylglycidate	γ-Undecalactone	Furaneol
10	0.1	0.5	0.1	1
20	1	5	0.5	3
30	3	8	1	5
---	25	20	10	---
---	75	35	15	---
---	150	50	20	---

3.4.2 Partition Coefficients

Following the procedure established by Bartelt (1997) the partition coefficients of *cis*-3-hexen-l-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate, γ-undecalactone and furaneol were determined. Five μL of each diluted flavor solution (Table 3.3) was injected into a sealed 10 mL sample vial by using a 10 μL Hamilton syringe (Reno, NA). The sample vials were equilibrated for an hour and then a DVB/Carboxen/PDMS Stableflex fiber was used to absorb the volatile compounds for 40 minutes at room

temperature. Further, the SPME fiber was injected into the GC by the autosampler. The detailed setup and the temperature programs of the GC for the five different flavor compounds were exactly the same as for the determination of the external standard calibration curves (see section 3.3.2).

3.5 Determination of Flavor Release from Emulsions

Combining *cis*-3-hexen-l-ol (0.375g/100g), α-ionone (0.375g/100g), ethyl-3-methyl-3-phenylglycidate (3.750g/100g), γ-undecalactone (0.500g/100g) and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (1.875g/100g) at these specific concentrations will give a distinct strawberry flavor. These five flavor compounds were added into ice cream mix at the ratio of 0.1% (v/v) to make strawberry flavored ice cream. In this study, the concentrations of five flavor compounds in the emulsion systems were the same as the concentrations of making strawberry ice cream.

3.5.1 Preparation of Flavor Solutions

Five stock solutions of each flavor were made as follows: 0.375 grams of *cis*-3-hexen-l-ol, 3.750 grams of ethyl-3-methyl-3-phenylglycidate, 0.375 grams of

α-ionone, 1.875 grams of 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 0.500 grams of γ-undecalactone were individually weighed into 50 mL clean capped sample vials and made to total 25 grams by methanol (Serial# A454-4, Fisher Scientific, Fair Lawn, NJ). These stock solutions were 4 times more concentrated than the solutions previously used for flavoring the ice cream mixes (see above) because for the experiment, the working solutions needed to be freshly prepared daily. The stock flavor solutions were tightly capped and kept in the refrigerator. Stock flavor solutions were warmed to room temperature and stirred for one hour before making dilutions to prepare working solution. Each diluted flavor solution was freshly made on the day of the experiment. The stock solutions were diluted in a 1:4 ratio as follows: 2 grams of *cis*-3-hexen-l-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate, γ-undecalactone and 2,5-dimethyl-4-hydroxy-3(2H)-furanone stock solutions were individually weighed into 15 mL capped test tubes and then made to total 8 grams with methanol (Serial# A454-4, Fisher Scientific, Fair Lawn, NJ).

3.5.2 Preparation of Emulsion Systems

There were six different kinds of emulsion systems (0% fat, 4% fat, 4% fat +

Simplesse®, 4% fat + Litesse®, 4% fat + Simplesse®/Litesse®, 10% fat) in this study (Table 3.4). Each emulsion was stirred in a 75ºC water bath for five minutes and then immediately homogenized twice by a hand-homogenizer (Fisher Scientific, Pittsburgh, PA). The emulsions were prepared freshly right before using.

Table 3.4 The formulation of emulsion systems (unit: grams)

	0%	4%	4% + S	4% + L	4% + S/L	10%
DI Water	99.5	94.63	87.33	87.33	87.33	87.33
Butter	---	4.87	4.87	4.87	4.87	12.17
Emulsifier	0.50	0.50	0.50	0.50	0.50	0.50
Simplesse®	---	---	7.30	---	3.65	---
Litesse®	---	---	---	7.30	3.65	---

3.5.3 Flavor Release from Emulsion Systems

The flavor release experiments of the five different flavor compounds (*cis*-3-hexen-l-ol, α-ionone, γ-undecalactone, ethyl-3-methyl-3-phenylglycidate, 2,5-dimethyl-4-hydroxy-3(2H)-furanone) from the six different emulsion systems (0% fat, 4% fat, 4% fat +

Simplesse®, 4% fat + Litesse®, 4% fat + Simplesse®/Litesse®, 10% fat) were conducted in four replications. Replications were based on emulsion systems, which mean each replication contained all six emulsion systems. The sequence of preparing each emulsion system was randomized and differed for each replication. Within each replication, the addition and measurement of flavor chemicals to the emulsion systems was randomized as well.

Five mL of each emulsion (0% fat emulsion or 4% fat emulsion or 4% + Simplesse® fat emulsion or 4% + Litesse® fat emulsion or 4% + Simplesse®/Litesse® or 10% fat) was individually pipetted into five 10 mL beakers, and then five μL of each flavor solution was added into the emulsion system, respectively. Three mL of the emulsion/ flavor solution was placed into a 10 mL sample vial and then immediately sealed with aluminum seals and septa. The actual amount of each flavor compound in each 10 mL sample vial was: 11250 ng of *cis*-3-hexen-l-ol, 11250 ng of α-ionone, 112500 ng of ethyl-3-methyl-3-phenylglycidate, 15000 ng of γ-undecalactone and 56250 ng of furaneol. The sealed sample vials were stirred for one hour to reach equilibrium. After equilibration, a DVB/ Carboxen/ PDMS Stableflex fiber was introduced into the sealed sample vial, and the fiber absorbed the volatile compound for 40 minutes at room temperature. The

detailed setup and the temperature programs of the GC for the five different flavor compounds were exactly the same as for the determination of the external standard calibration curves (see section 3.3.2). The actual amounts of flavor released from the emulsion systems were calculated by the equations of partition coefficients and external standard curves.

3.6 Data Analysis

3.6.1 Partition Coefficient Data

According to Bartelt (1997), the partition coefficient of the flavor compound for the SPME fiber was calculated as following:

$$\text{HS Concentration} = \frac{\text{Analyte in the bottle (ng)} - \text{Analyte on the fiber (ng)}}{\text{Volume of the bottle (mL)}} \quad \textbf{\{eq.1\}}$$

$$K = \frac{\text{Analyte on fiber (ng)/Volume of the fiber (mL)}}{\text{HS concentration (ng/mL)}} \quad \textbf{\{eq.2\}}$$

*HS Concentration = Headspace Concentration

*K = Partition Coefficient

For equation 1, the amount of analyte placed into the bottle was based on the concentrations (Table 3.3) and the volume of the bottle was given (10 mL). The analyte amount on the fiber was calculated based on the regression lines of the external standard curves, in order to determine the headspace concentration. The partition coefficient

equations were derived from plotting the HS concentration (ng/mL) as the independent variable on the X-axis and amount (ng) on fiber as the dependent variable on the Y-axis (Figures 3.7-3.12).

3.6.2 Calculation of the Percentage of Amount Released from Emulsions

The GC results are the area count of each peak, which correspond to the flavor chemicals in the emulsion. The area counts were inserted into the equations of the external standard curves to determine the actual amounts of each flavor chemical on the fiber. Knowing the actual amounts on the fiber allowed the calculation of the HS concentration through the partition coefficient equation, which, as describe above, was determined by plotting the HS concentration (ng/mL) on the X-axis and the amount (ng) on fiber on the Y-axis. With the HS concentration known, the actual amount of flavor compound in the headspace can be calculated by multiplying the HS concentration by the headspace volume. Further, the percentage of flavor released from the emulsion system was then calculated as actual amount in the headspace divided by the amount added into emulsion and then multiplied by 100 to get percent released (Tables 3.5-3.10).

An example of the calculation is shown here:

Three milliliters of 10% fat emulsion, which contained 11,250 ng of *cis*-3-hexen-1-ol, was put into a capped 10 mL sample vial. The headspace concentration of the amount of *cis*-3-hexen-1-ol released from 10% fat emulsion was determined by GC. The GC peak yielded an area count equal to 222,515 (Table 3.5). This area count was inserted into the regression equation of the external standard curve to determine the actual amount of *cis*-3-hexen-1-ol on the fiber. The external standard equation (y= 10972x – 1571.7) of *cis*-3-hexen-1-ol was derived by using amounts (ng) injected into GC as the independent variable (X-axis) and peak area counts as the dependent variable (Y-axis) (Figure 3.1). The calculation ((222515 + 1571.7)/10972 = 20.42) showed that the actual amount of *cis*-3-hexen-1-ol on the fiber was 20.42 ng. Knowing the actual amounts of *cis*-3-hexen-1-ol on the fiber allowed the calculation of the HS concentration through the partition coefficient equation. The partition coefficient equation (y= 2.8054x – 5.9822) of *cis*-3-hexen-1-ol was derived from plotting the HS concentration (ng/mL) as the independent variable on the X-axis and amount (ng) on fiber as the dependent variable on the Y-axis (Figure 3.7). From this calculation ((20.42 + 5.9822)/2.8054 = 9.41) the HS concentration of cis-3-hexen-1-ol was determined to be 9.41 ng/mL. The actual amount of *cis*-3-hexen-1-ol in the headspace was further calculated by multiplying the HS

concentration by the headspace volume. The headspace volume was the total volume of the sample vial minus the volume of the emulsion (10 – 3 = 7 mL). Thus (9.41 ng/mL * 7 mL = 65.87 ng), the actual amount of *cis*-3-hexen-1-ol in the headspace was 65.87 ng. Furthermore, the percentage of *cis*-3-hexen-1-ol released from 10% emulsion system was then calculated as actual amount in the headspace divided by original amount added into emulsion (65.87/ 11250 = 0.00585) and then multiplied by 100 to get percent released (0.00585 * 100 = 0.585 %). The result showed that there was 0.585% of *cis*-3-hexen-1-ol released from 10% emulsion system.

3.6.3 Statistical Analysis

All data were analyzed by using the Statistical Analysis System (SAS) program (SAS 1999). For the partition coefficient, ANOVA (analysis of variance) was used to determine significant differences between K values and the amount of flavor compound in the headspace. For the determination of flavor released from the emulsion systems, the significant differences between different fat treatments were analyzed by using the General Linear Model (GLM) procedure in SAS.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Partition Coefficient Analysis

For getting accurate quantitative analysis, it is important to determine the distribution of each flavor compound between the SPME fiber and the sample matrix (Yang and Peppard 1999). The partition coefficient (K value) between SPME fiber (50/30 μm DVB/ Carboxen™/ PDMS Stableflex) and the five strawberry flavor compounds (*cis*-3-hexen-1-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate, γ-undecalactone and furaneol) were determined by following Bartelt's (1997) method. The concentration levels of five flavor compounds used for the determination of the partition coefficients are shown in Table 3.3. The determination of significant differences between K values and the amount of flavor compound in the headspace was analyzed by ANOVA (analysis of variance). As was also found by Bartelt (1997), a concentration dependent K value was observed for some of the compounds.

The results showed that different amounts (50, 100, 150 ng) of *cis*-3-hexen-1-ol in the headspace did not significantly influence the K value ($P = 0.1644$) (Table 4.1), indicating

that that the K value of *cis*-3-hexen-1-ol is independent of the analyte's concentration.

At low concentration levels (0.5, 5, 15 ng) of α-ionone (Table 4.2a), the K value significantly increased at the lowest concentration. Comparing these three amount levels of α-ionone, the K value for the low amount (0.5 ng) was 5.5 to 7 times higher than that of the medium (5 ng) and high amounts (15 ng), respectively. However, there was no significant difference between the medium and high amounts.

Flavor compounds can attach to the fiber through two mechanisms. First, flavor compounds attach to the fiber through binding to sites on the "outside" of the fiber that are specifically active, i.e. have a high affinity for the chemical, by adsorption. Second, when these active binding sites on the fiber are all occupied by flavor compounds, additional molecules are attached to the fiber via a normal absorption mechanism, which means the compounds need to move to the inner layers of the fiber. When this situation happens, the K value appears to be concentration dependent, with smaller analyte amounts having larger K values (Lord and Pawliszyn 2000).

In this research, the sample vials were silanized before use, so it is unlikely that the analyte attached to the glass of the sample vials. In addition, if analyte absorption by the sample vial were the reason for differing K values, the K value should be lower for lower

concentrations, which they were not.

Table 4.2a Partition coefficient data of α-ionone at low concentration levels (P < 0.05)

Amount in the headspace (ng)	Mean K[1] value
0.5	22.06[a 2]
5	4.25[b]
15	3.94[b]

[1]K value= partition coefficient

[2]Means sharing the same letter do not differ at P<0.05

At high concentration levels (125, 375, 750 ng) of α-ionone (Table 4.2b), the opposite phenomenon was observed. The K value significantly decreased at the low amount level (125 ng). On the other hand, similar to before, there was no significant difference between the medium (375 ng) and high (750 ng) amount levels. This result cannot be explained by an apparent concentration dependence effect due to different absorption mechanisms, because the amounts of analyte in the headspace were much larger than those at the low concentration levels. The true K value is calculated as the concentration of analytes on the fiber divided by the concentration of analytes in the headspace.

Table 4.2b Partition coefficient data of α-ionone in high concentration levels (P < 0.05)

Amount in the headspace (ng)	Mean K[1] value
125	6.63[b 2]
375	12.56[a]
750	11.19[a]

[1]K value= partition coefficient

[2]Means sharing the same letter do not differ at P<0.05

The results of the *cis* isomer of ethyl-3-methyl-3-phenylglycidate in the low concentration levels (2.5, 25, 40 ng) showed that the amount of the *cis* isomer in the headspace did not influence the K value (P = 0.0648) (Table 4.3a). However, at high concentration levels (100, 175, 250 ng) of the *cis* isomer, the K value of the medium amount was significantly higher than that of the high amount level followed by the low amount level (Table 4.3b).

Table 4.3b Partition coefficient data of ethyl-3-methyl-3-phenylglycidate *cis* isomer in high concentration levels (P < 0.0001)

Amount in the headspace (ng)	Mean K[1] value
100	5.59[c 2]
175	12.68[a]
250	10.57[b]

[1]K value= partition coefficient

[2]Means sharing the same letter do not differ at P<0.05

At the low concentration levels (2.5, 35, 40 ng) of the *trans* isomer, the K value of the low amount level was significantly higher than that at the high amount level followed by the medium amount level (Table 4.4a). Comparing low amount level with the other two amount levels, the reason why the low amount level had higher K values is because the apparent concentration dependence effect. However, the medium amount level of the *trans* isomer had a lower K value than the high amount level. This slight difference might due to the competition effect between *cis* and *trans* isomers. The *trans* isomer had poorer affinity toward the SPME fiber, so the *cis* isomer more readily occupied the active site by adsorption mechanism or got into the SPME fiber via absorption. This competition effect might lead to minor differences in the K values for medium and high amount levels.

Table 4.4a Partition coefficient data of ethyl-3-methyl-3-phenylglycidate *trans* isomer in low concentration levels (P < 0.05)

Amount in the headspace (ng)	Mean K[1] value
2.5	1.98[a 2]
25	1.17[c]
40	1.54[b]

[1]K value= partition coefficient

[2]Means sharing the same letter do not differ at P<0.05

At the high concentration level (100, 175, 250 ng) of the *trans* isomer (Table 4.4b), the K value of the low amount level (100 ng) was significantly lower than that of the medium (175 ng) and the high (250 ng) amount levels. There was no significant difference for the K value between the medium and the high amount levels. Jung and Ebeler (2003) observed that flavor compounds have larger K value when they have a greater affinity toward the fiber. Also, Yang and Peppard (1999) indicated that more polar compounds have lower affinity toward non-polar fiber coatings. Thus, our results would indicate that the *cis* isomer had a higher affinity toward fiber and also more non-polar characteristics than the *trans* isomer.

Table 4.4b Partition coefficient data of ethyl-3-methyl-3-phenylglycidate *trans* isomer in high concentration levels (P < 0.0001)

Amount in the headspace (ng)	Mean K[1] value
100	1.25[b 2]
175	2.72[a]
250	2.99[a]

[1]K value= partition coefficient

[2]Means sharing the same letter do not differ at P<0.05

At the low concentration level (0.5, 2.5, 5 ng) of γ-undecalactone, the K value of the low amount was significantly lower than at the high amount followed by the medium amount (Table 4.5a). Comparing the K values between medium (2.58) and high (1.60) amount levels, the K value of medium amount level was higher than that of the high amount level. There was an apparent concentration dependence effect between medium and high amount levels. However, the K value of the low amount level (0.15) was much lower than the others. This was because of the poor affinity of γ-undecalactone toward SPME fiber and a too small amount of analyte at the low amount level. Comparing the K value of α-ionone (Table 4.2a) and γ-undecalactone (Table 4.5a) at same amount level (0.5 ng in the headspace), the K value of α-ionone was 22.06 but the K value of

γ-undecalactone was 0.15. This indicated that γ-undecalactone had very poor affinity toward the SPME fiber. On the other hand, at the high concentration level (50, 75, 100 ng) of γ-undecalactone (Table 4.5a), the K value was not significantly influenced by the differing amount of analyte in the headspace (P = 0.1089).

Table 4.5a Partition coefficient data of γ -undecalactone in low concentration levels (P < 0.0001)

Amount in the headspace (ng)	Mean K[1] value
0.5	0.15[c 2]
2.5	2.58[a]
5	1.60[b]

[1]K value= partition coefficient

[2]Means sharing the same letter do not differ at P<0.05

At this point, there is no logical explanation for larger K values at higher amount levels such as the data of α-ionone, ethyl-3-methyl-3-phenylglycidate *cis* and *trans* isomers at the high concentration level. There are plenty of research papers describing the partition coefficient relationship between SPME fibers and flavor compounds. However, only very few papers explored the partition coefficient between SPME fibers and specific flavor

compound at different concentration levels. From the limited number of research papers, the results cannot sufficiently explain the phenomenon because of the narrow range of concentration levels used by other researchers compared to the ranges used in our study. For example, Bartelt (1997) determined the partition coefficient between 1 to 25 ppb of 71 analytes and Roberts (2000) determined dimethyltrisulfide between 1 to 4 ppm, 3-methylbutanal between 3.75 to 15 ppm and 2-isobutyl-3-methoxy-pyrazine between 6 to 24 ppm. In our study, the GC injections of *cis*-3-hexen-1-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate and γ-undecalactone were randomized across flavor chemicals and concentration levels, and were all done with the same SPME fiber. Thus, the K value differences cannot be attributed to some pattern of injection sequences or be due to different fibers. Also, the sample vials were silanized before use, so it is unlikely that the analyte attached to the glass of the sample vials. If we assume that equilibrium was not achieved during the 40 minutes extraction period, due to too high concentrations, the K value should be lower for these higher concentrations. However, the results showed higher K values at these higher concentration levels. Based on the evidence, there should not be any bias caused by the experimental design and operations. However, the complex fiber coating of DVB/ Carboxen™/ PDMS Stableflex might be the reason for this

phenomenon of an apparent concentration dependent K-value. In this experiment, the partition coefficient was measured between specific flavor compound and the SPME fiber. Because in the literature where partition coefficients at different concentrations were measured, only single coating SPME fibers such as PDMS (Bartelt 1997; Martos and Pawliszyn 1997; Roberts and others 2000; Jung and Ebeler 2003) were used. Thus, the partition coefficient between complex coating SPME fiber and flavor compounds remains unknown. The larger K values at higher amounts might be due to the complex partition coefficient between DVB/ Carboxen™/ PDMS Stableflex and ethyl-3-methyl-3-phenylglycidate *cis* and *trans* isomers and α-ionone at high concentration levels.

The ANOVA result of furaneol (Table 4.6) showed that there were significant differences among the K value between low (5 ng), medium (15 ng) and high (25 ng) amounts of furaneol. The highest K value was determined at the high amount level followed by the medium amount and then the low amount level. Furaneol has very different chemical characteristic compared to the other four compounds. It is a water-soluble and more polar flavor compound (Pyysalo and other 1979; Bauer and others 1997). From the low K values, it can be concluded that furaneol has very low

affinity to the SPME fiber. Also, the K values for three different amount levels gradually increased with increasing amount levels. This might be due to the analytes not having reached equilibrium and a relatively faster uptake from headspace at high amount level. Shiery (1999) confirmed that polar chemical compounds are more difficult to extract by SPME fibers and take longer time to reach equilibrium.

Table 4.6 Partition coefficient data of furaneol (P < 0.05)

Amount in the headspace (ng)	Mean K[1] value
5	0.04[c 2]
15	0.29[b]
25	0.72[a]

[1]K value= partition coefficient

[2]Means sharing the same letter do not differ at P<0.05

These results showed that different chemical compounds have different partition coefficients with the SPME fiber. Since only one chemical compound was measured at a time, there could not have been any competition effect among the chemical compounds. Also, the R square of partition coefficient curve of *cis*-3-hexen-1-ol, α-ionone and

ethyl-3-methyl-3-phenylglycidate were higher than 0.95 and the R square of γ-undecalactone and furaneol were higher than 0.88 (Figure 3.7-3.12). The R squares were in the acceptable linear range, so these partition coefficient curves were used for the further quantitative experiment of measuring flavor released from emulsion systems.

4.2 Flavor Released From Emulsion Systems

Flavors are detected by their release into the headspace inside the mouth when people consume food products. The headspace flavors are further transported from mouth to the olfactory glands, and then human body has the sense of smell (de Roos 1997). In this study, the percentage of five strawberry flavor compounds (*cis*-3-hexen-l-ol, α-ionone, γ-undecalactone, ethyl-3-methyl-3-phenylglycidate, furaneol) released from six emulsion systems (0% fat, 4% fat, 4% fat + Simplesse®, 4% fat + Litesse®, 4% fat + Simplesse®/Litesse®, 10% fat) were measured. Significant differences of the percentage of flavor released from the emulsion systems between different fat treatments were analyzed by using the General Linear Model (GLM) procedure in SAS.

4.2.1 *Cis*-3-hexen-1-ol

The percentage of *cis*-3-hexen-1-ol released is shown in Table 4.7. The release of *cis*-3-hexen-1-ol from the 10% fat emulsion was significantly lower than that from the 4% and 0% fat emulsions. The percentage of *cis*-3-hexen-1-ol released from emulsion systems increased with a decrease of fat levels. For example, the average percentage of *cis*-3-hexen-1-ol released from 10% fat was 0.59, while the percentage released from 4% and 0% fat was 0.72 and 0.80, respectively. However, there was no significant difference between the 0% and the 4% fat emulsion, or the 4% with Simplesse emulsion. However, the addition of Litesse, either alone or in combination with Simplesse, significantly decreased its release compared to the 0% emulsion. The reason is because of *cis*-3-hexen-1-ol is slightly soluble in water, and it is a more polar compound comparing to the other volatile compounds (Burdock 1994). de Roos and Wolswinkel (1994) showed that *cis*-3-hexen-1-ol has similar correction factor in water and milk sample. Also, de Roos (1997) confirmed that *cis*-3-hexen-1-ol is a water soluble compound, and its volatility is barely influenced by the fat levels in emulsion systems. On the other hand, Miettinen and others (2002) showed that release of *cis*-3-hexen-1-ol is significant influenced by fat content in strawberry ice creams with three different fat contents (0, 9

and 18%).

From the aspect of fat mimetics, there was no significant difference between 4% fat, 4% fat + Simplesse®, 4% fat + Litesse® and 4% fat + Simplesse®/Litesse®. However, percent release of *cis*-3-hexen-1-ol from 4% fat + Litesse® and 4% fat + Simplesse®/Litesse® were more similar to the 10% fat than that of the 4% fat + Simplesse® emulsion. Fabre and others (2002) showed that *cis*-3-hexen-1-ol had poor affinity to protein. This might be the reason why Litesse®, which is a carbohydrate-based fat mimetic, contributed better flavor profile than Simplesse®, a protein-based fat mimetic. This result indicated that using Litesse® or a Simplesse® /Litesse® mix as a fat mimetic would contribute better flavor profile for reducing the release of *cis*-3-hexen-1-ol from emulsion system than Simplesse®.

Table 4.7 The percentage of *cis*-3-hexen-1-ol released from six emulsion systems (P < 0.05)

Percent fat (%)	Flavor released into HS[1] (%)
0[2]	0.80[a 3]
4	0.72[ab]
4 + S	0.73[ab]
4 + L	0.69[b]
4 + S/L	0.69[b]
10	0.59[c]

[1]HS=headspace; [2]0=0% fat; 4=4% fat; 10=10% fat; 4+S=4% fat with Simplesse®; ; 4+L=4% fat with Litesse®; 4+S/L=4% fat with Simplesse® and Litesse®
[3]Means sharing the same letter do not differ at P<0.05

4.2.2 α-ionone

The amount of α-ionone released into the headspace from 0% fat was much higher than that of the other emulsion systems (Table 4.8). For instance, the average of flavor released from the 0% fat was 85 times higher than that from the 4% fat emulsion and 200 times higher than that from the 10% fat emulsion. This large difference between the no-fat versus fat-containing emulsions masked any potential differences amongst the fat-containing emulsions. To eliminate this problem, the percentages of α-ionone released

from the emulsion systems were transformed into logarithmic values. The results showed that the α-ionone released from the emulsion systems significantly decreased with an increasing fat level. For instance, the mean logarithmic value of the percentage of α-ionone released from 0%, 4% and 10% fat emulsions was 0.44, -1.49 and -1.86, respectively. This is because α-ionone is a lipophilic compound and is strongly influenced by the fat content in the emulsion systems. de Roos and Wolswinkel (1994) showed that 98% of α-ionone remained in an oil-in water emulsion, but only 45% remained in plain water. Further, de Roos (1997) confirmed this by adding 1% olive oil into CMC (carboxymethyl cellulose) solution, showing that the addition of 1% olive oil dramatically decreased α-ionone release from a CMC emulsion system.

However, there was no significant difference between 4% fat, 4% fat + Simplesse®, 4% fat + Litesse®, 4% fat + Simplesse®/Litesse®, and all four were significantly different from the 10% fat system, indicating that using Simplesse® and/or Litesse® as fat mimetics does not increase the retained amount of α-ionone in emulsion systems.

Table 4.8 The percentage and log percent of α-ionone released from six emulsion systems (P < 0.0001)

Percent fat (%)	Flavor released into HS (%)	Flavor released into HS[1] (log %)
0[2]	2.81	0.44[a 3]
4	0.033	- 1.49[b]
4 + S	0.032	- 1.55[b]
4 + L	0.028	- 1.56[b]
4 + S/L	0.031	- 1.50[b]
10	0.014	- 1.86[c]

[1]HS=headspace; [2]0=0% fat; 4=4% fat; 10=10% fat; 4+S=4% fat with Simplesse®; ; 4+L=4% fat with Litesse®; 4+S/L=4% fat with Simplesse® and Litesse®
[3]Means sharing the same letter do not differ at P<0.05

4.2.3 Ethyl-3-methyl-3-phenylglycidate

The two isomers of ethyl-3-methyl-3-phenylglycidate differ in their release from the different emulsion systems. The percentage of the *cis* isomer released from the emulsion systems significantly decreased with increasing fat content (Table 4.9). The highest percentage of the *cis* isomer was released from the 0% fat mix (0.77%) followed by the 4% (0.14%) and the 10% fat mix (0.07%).

Table 4.9 The percentage of ethyl-3-methyl-3-phenylglycidate *cis* isomer released from six emulsion systems (P < 0.0001)

Percent fat (%)	Flavor released into HS[1] (%)
0[2]	0.77[a 3]
4	0.14[b]
4 + S	0.15[b]
4 + L	0.14[b]
4 + S/L	0.13[b]
10	0.07[c]

[1]HS=headspace; [2]0=0% fat; 4=4% fat; 10=10% fat; 4+S=4% fat with Simplesse®; ; 4+L=4% fat with Litesse®; 4+S/L=4% fat with Simplesse® and Litesse®
[3]Means sharing the same letter do not differ at P<0.05

The amount of the *trans* isomer released into the headspace from the 0% fat mix was more than 10 times higher than that from the other emulsion systems. This caused problems in determining differences among the other emulsion systems, so the percentages of the *trans* isomer released from the emulsion systems were transformed into logarithmic values. As seen for the *cis* isomer, the percentage of the *trans* isomer released from the emulsion systems significantly decreased with increasing fat levels (Table 4.10). For example, the mean logarithmic value of the percentage of the *trans*

isomer released from the 0% system was highest (-0.06), followed by the 4% (-1.16) and the 10% (-1.42) system. This fat level dependent effect is due to the polarity of ethyl-3-methyl-3-phenylglycidate. Ethyl-3-methyl-3-phenylglycidate is a lipophilic compound, which can dissolve well in organic solvents, but dissolves poorly in water. Kinsella (1990) mentioned that fat not only plays an important role for the flavor profile, but it acts as a flavor carrier as well as controls flavor release from the food matrix. Hydrophobic compounds would retain well in higher fat content emulsion systems compared to hydrophilic compounds. Also, Loeb (2004) confirmed that ethyl-3-methyl-3-phenylglycidate is perceived stronger in 10% strawberry ice cream than in 4% fat ice creams.

Table 4.10 The percentage and log percent of ethyl-3-methyl-3-phenylglycidate *trans* isomer released from six emulsion systems (P < 0.0001)

Percent fat (%)	Flavor released into HS (%)	Flavor released into HS[1] (log %)
0[2]	0.87	- 0.06[a 3]
4	0.070	- 1.16[b]
4 + S	0.076	- 1.12[b]
4 + L	0.073	- 1.14[b]
4 + S/L	0.069	- 1.16[b]
10	0.038	- 1.42[c]

[1]HS=headspace; [2]0=0% fat; 4=4% fat; 10=10% fat; 4+S=4% fat with Simplesse®; ; 4+L=4% fat with Litesse®; 4+S/L=4% fat with Simplesse® and Litesse®
[3]Means sharing the same letter do not differ at P<0.05

There were no significant difference between 4% fat, 4% fat + Simplesse®, 4% fat + Litesse® and 4% fat + Simplesse®/Litesse® neither for the *cis* nor the *trans* isomer indicating that fat mimetics do not influence *cis* and *trans* isomer release from emulsion systems. This result is in agreement with Liou's (2006) results that the perception of ethyl-3-methyl-3-phenylglycidate (candy flavor) does not differ in strawberry ice creams manufactured with or without fat mimetics.

4.2.4 γ-undecalactone

The amount of γ-undecalactone released into the headspace from the 0% fat mix was much higher than that from the other emulsion systems, such as being 130 times higher than that from the 4% fat emulsion and 330 times higher than that from the 10% fat emulsion (Table 4.11). This again caused problems with identifying differences among the other emulsion systems, so the percentages of γ-undecalactone released from the emulsion systems were transformed into logarithmic values. The largest mean logarithmic value of the percentage of γ-undecalactone released was measured for the 0% fat emulsion (-0.46) followed by the release from the 4% (-2.59) mix and the 10% (-3.05) mix. The log percentages of γ-undecalactone released from emulsion systems significantly depended on the level of fat. The higher the fat content the lower is the release (Table 4.11). Fat has been reported to influence the perception of flavor in food products (Plung and Haring 1994), and low fat foods have been shown to often have poor flavor quality (Giese 1994). It is also well known that γ-undecalactone is a highly lipophilic compound (Guth and others 2001). It poorly dissolves in water but has good solubility in organic solvents (Bauer and others 1997). This highly lipophilic characteristic strongly influences γ-undecalactone's release from emulsion systems with

different fat levels.

The results also indicated that Litesse® performed significantly better than Simplesse® in retaining γ-undecalactone in the emulsion system. The logarithmic value of the percentage of γ-undecalactone released from 4% + Litesse® was -2.99 and from 4% + Simplesse® it was -2.57. In addition, no differences were found in the release of γ-undecalactone from the 4% + Litesse® mix and the 10% emulsion system, which means 4% + Litesse® can replace 10% fat for the flavor release of γ-undecalactone.

Table 4.11 The percentage and log percent of γ-undecalactone released from six emulsion systems (P < 0.0001)

Percent fat (%)	Flavor released into HS (%)	Flavor released into HS[1] (log %)
0[2]	0.35	- 0.46[a 3]
4	0.0026	- 2.59[bc]
4 + S	0.0030	- 2.57[b]
4 + L	0.0014	- 2.99[cd]
4 + S/L	0.0026	- 2.60[bc]
10	0.0011	- 3.05[d]

[1]HS=headspace; [2]0=0% fat; 4=4% fat; 10=10% fat; 4+S=4% fat with Simplesse®; ; 4+L=4% fat with Litesse®; 4+S/L=4% fat with Simplesse® and Litesse®
[3]Means sharing the same letter do not differ at P<0.05

4.2.5 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol™)

Because of the difference in polarity, furaneol had very different results compared to the other four flavor compounds. The percentage of furaneol released from the emulsion systems significantly increased with increasing fat levels (Table 4.12). The highest percentage of furaneol released was 0.013 from 10% fat emulsion and following by the 4% emulsion (0.012) and 0% (0.010). Even though there were significant differences

between different levels of fat content, the differences were very small (around 0.001%). This is because furaneol is a highly polar and water-soluble flavor compound and not greatly influenced by different fat levels (Pyysalo and other 1979; Bauer and others 1997). This polar characteristic allows furanol to maintain similar flavor release and thus perception even in very low fat or non-fat emulsion systems.

The result also indicated there were no significant differences between 4% fat, 4% fat + Simplesse®, 4% fat + Litesse®, 4% fat + Simplesse®/Litesse®, but the 4% + Simplesse® was more similar to the 10% fat emulsion than the other emulsions systems in its capability of retaining furaneol. This result is in agreement with Liou's (2006) research that 4% + Simplesse® performed more like 10% ice cream than 4% fat + Litesse® in regard to the perception of furaneol flavor.

Table 4.12 The percentage of furaneol released from six emulsion systems (P < 0.0001)

Percent fat (%)	Flavor released into HS[1] (%)
0[2]	0.0103[c 3]
4	0.0115[b]
4 + S	0.0123[ab]
4 + L	0.0115[b]
4 + S/L	0.0115[b]
10	0.0128[a]

[1]HS=headspace; [2]0=0% fat; 4=4% fat; 10=10% fat; 4+S=4% fat with Simplesse®; ; 4+L=4% fat with Litesse®; 4+S/L=4% fat with Simplesse® and Litesse®
[3]Means sharing the same letter do not differ at P<0.05

In summary, the five strawberry flavor compounds were significantly influenced by different fat contents (0%, 4% and 10%). The percentages of *cis*-3-hexen-1-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate and γ-undecalactone released from emulsion systems decreased with increasing fat contents. However, furaneol decreased with decreasing fat content. Regarding the fat mimetics, α-ionone and ethyl-3-methyl-3-phenylglycidate were not influenced by adding Simplesse® or Litesse®. However, release of *cis*-3-hexen-1-ol and γ-undecalactone was similar to the 10% fat for the 4% + Litesse®

formulation, and furaneol release was similar to 10% fat for the 4% + Simplesse® formulation.

CHAPTER 5

SUMMARY AND CONCLUSION

The flavor release of five strawberry flavor compounds (*cis*-3-hexen-1-ol, α-ionone, ethyl-3-methyl-3-phenylglycidate, γ-undecalactone, furaneol) were determined in six different emulsion systems (0% fat, 4% fat, 4% fat + Simplesse®, 4% fat + Litesse®, 4% fat + Simplesse®/Litesse®, 10% fat). From the aspect of fat levels, the release of *cis*-3-hexen-1-ol, α-ionone, γ-undecalactone and ethyl-3-methyl-3-phenylglycidate was significantly decreased with increasing fat levels. For example, the percentage of flavor release from 0% fat was higher than from 4% fat followed by 10% fat. The reason for decreasing release with increasing fat level is that these four compounds are rather non-polar compounds that are retained by the fat in the emulsion. On the other hand, furaneol is a more polar compound, and it had the highest percentage of flavor release from 10% fat then 4% fat followed by 0% fat.

From the aspect of fat mimetics and flavor release, there was no significant difference between flavor release from the six emulsion systems for α-ionone and ethyl-3-methyl-3-phenylglycidate. However, the flavor release of *cis*-3-hexen-1-ol and

γ-undecalactone was more similar to 10% fat when Litesse® was used as the fat mimetic in the 4% ice cream mix. On the contrary, the flavor release of furaneol was more similar to 10% fat when Simplesse® was used as the fat mimetic.

These results provide useful references for formulating flavorings for ice cream with reduced fat contents. However, there are many more attributes that need to be considered when formulating low fat ice cream, such as mouthfeel, melting time and texture. Thus it would be important to choose a fat mimetic that can not only provide a good flavor profile but also match other important ice cream attributes.

APPENDICES

Appendix A Partition Coefficient Data

Table 4.1 Partition coefficient data of *cis*-3-hexen-1-ol (P=0.1644)

Amount in the headspace (ng)	Mean K value
50	2.10
100	2.66
150	3.24

Table 4.3a Partition coefficient data of ethyl-3-methyl-3-phenylglycidate *cis* isomer in low concentration levels (P=0.0648)

Amount in the headspace (ng)	Mean K value
2.5	6.37
25	5.04
40	6.48

Table 4.5b Partition coefficient data of γ -undecalactone in high concentration levels (P=0.1089)

Amount in the headspace (ng)	Mean K value
50	0.79
75	0.94
100	1.18

Appendix B Standard Curves and Partition Coefficient Curves

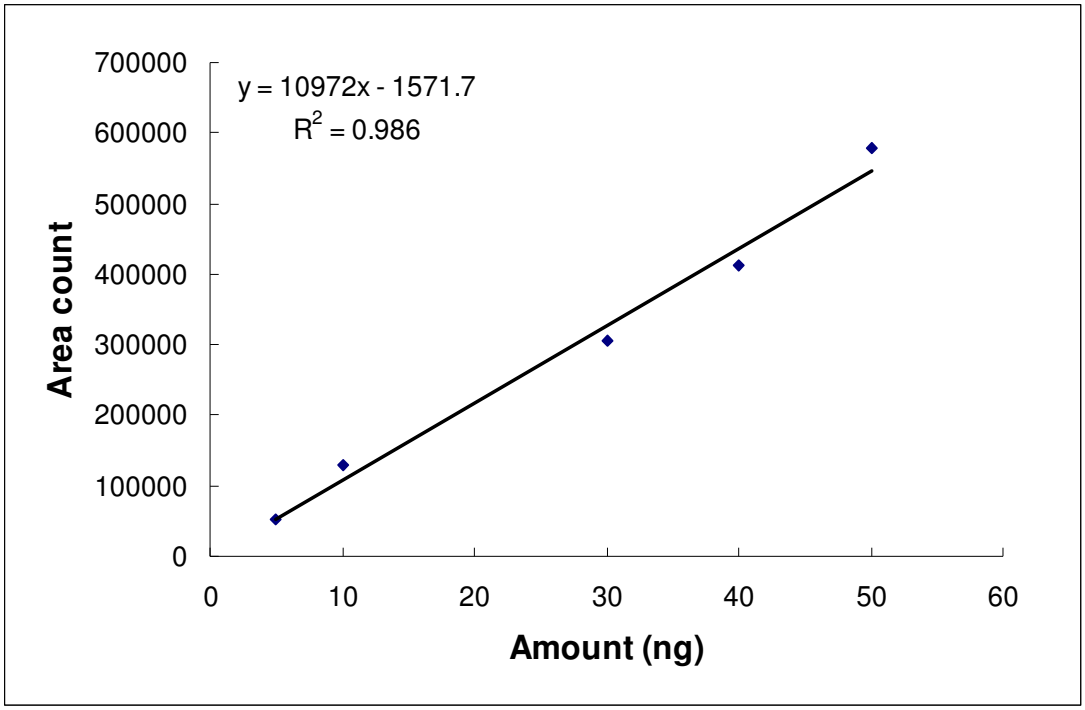

Figure 3.1 External standard curve of *cis*-3-hexen-1-ol

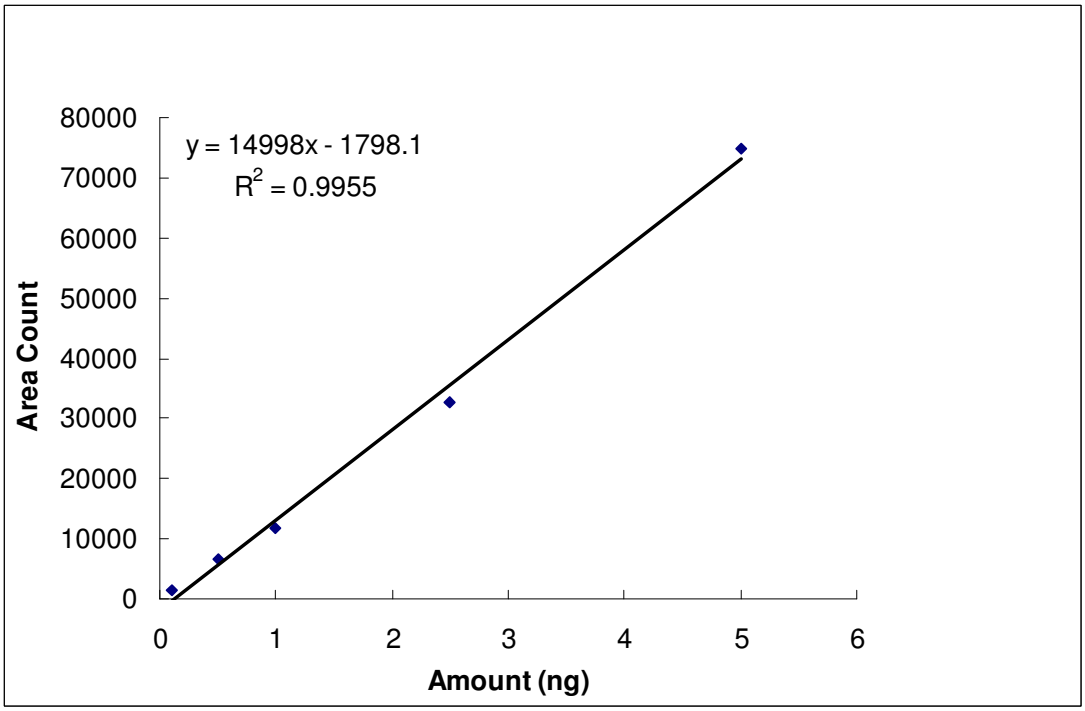

Figure 3.2a External standard curve of α-ionone (Low conc. level)

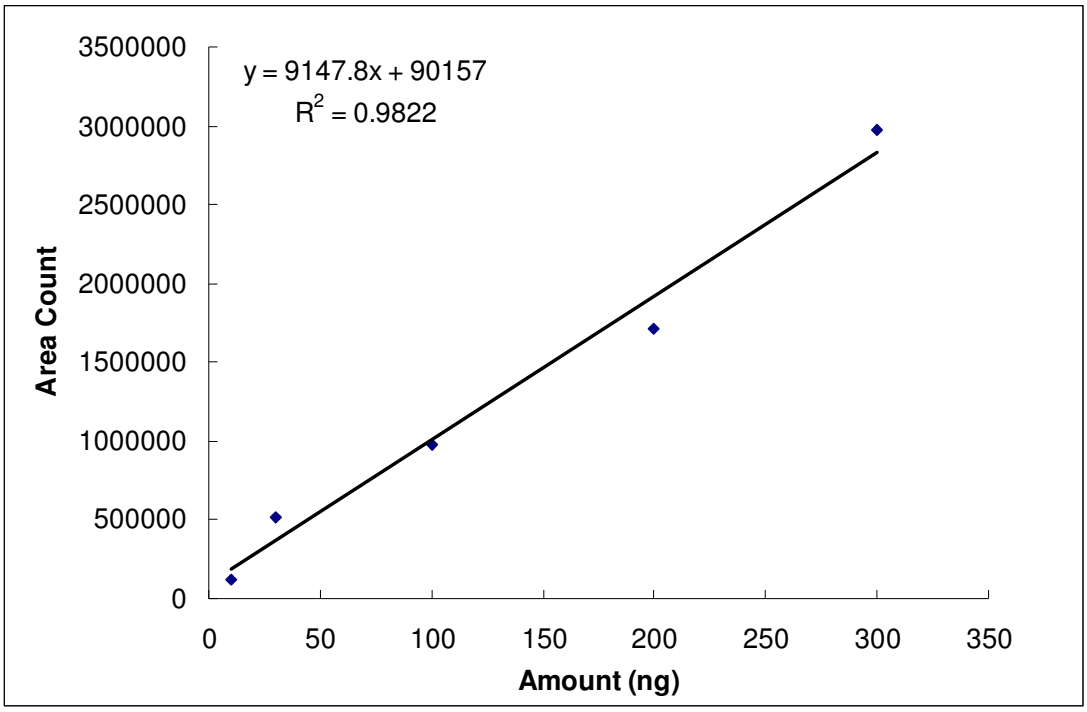

Figure 3.2b External standard curve of α-ionone (High conc. level)

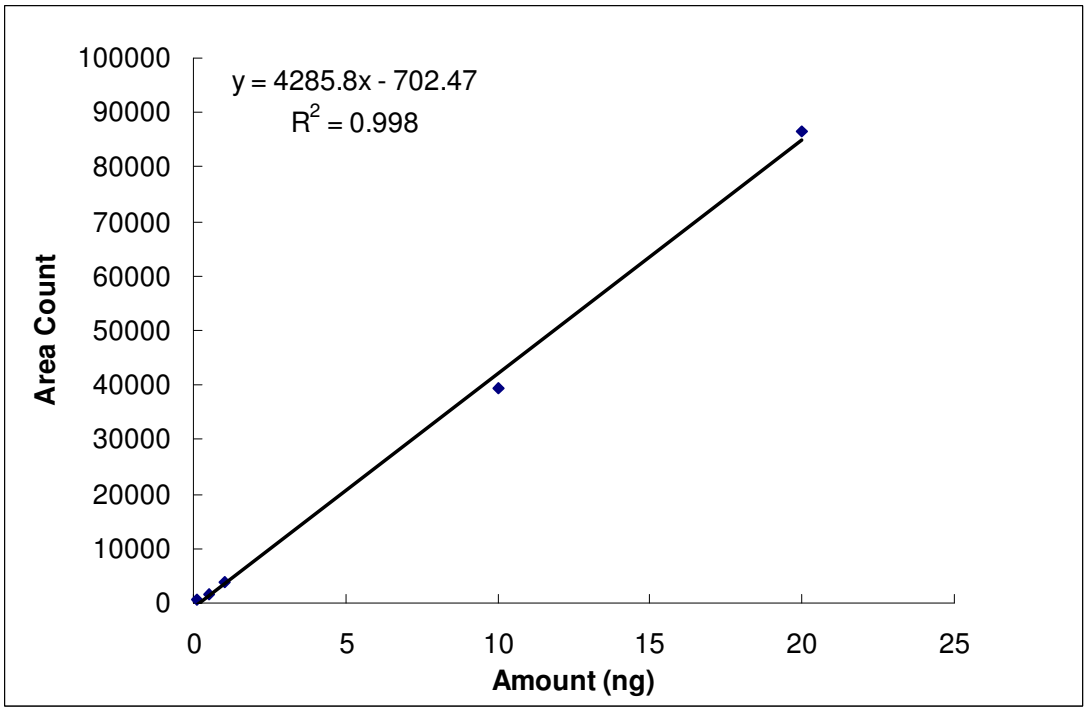

Figure 3.3a External standard curve of ethyl-3-methyl-3-phenylglycidate *cis* isomer (Low conc. level)

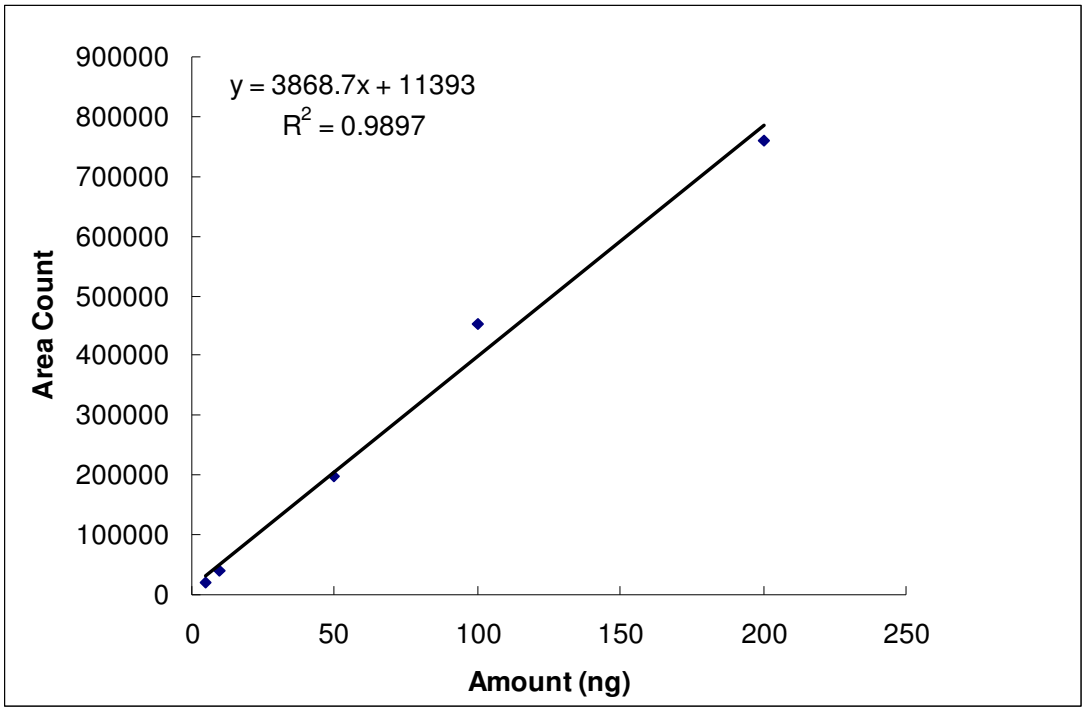

Figure 3.3b External standard curve of ethyl-3-methyl-3-phenylglycidate *cis* isomer (High conc. level)

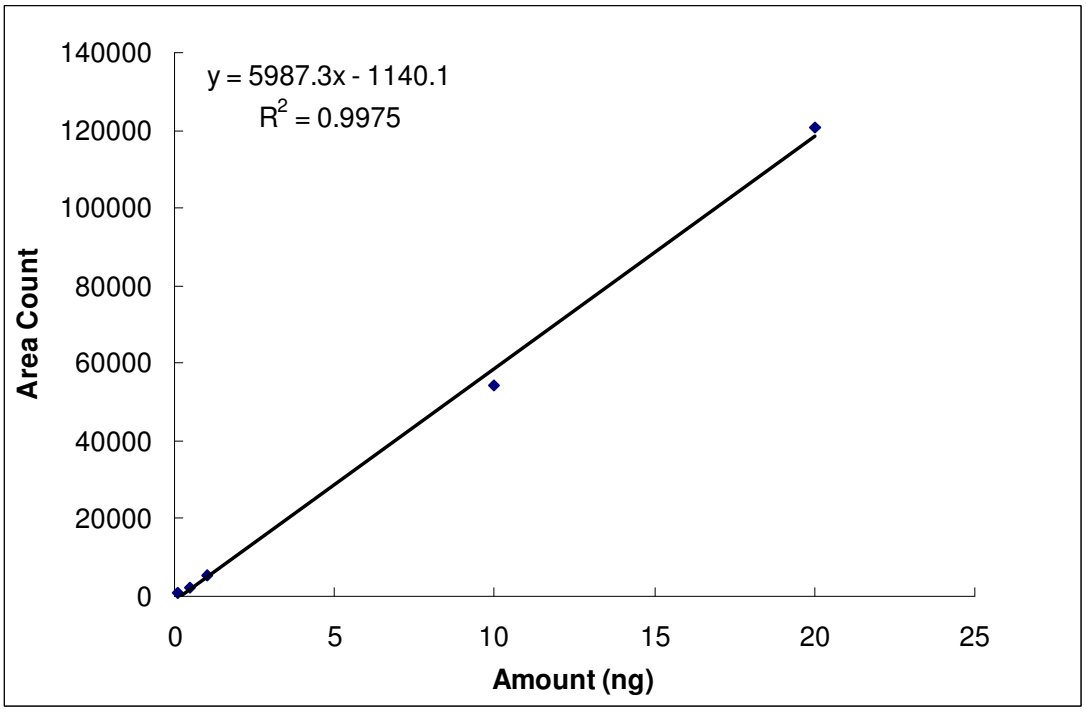

Figure 3.4a External standard curve of ethyl-3-methyl-3-phenylglycidate *trans* isomer (Low conc. level)

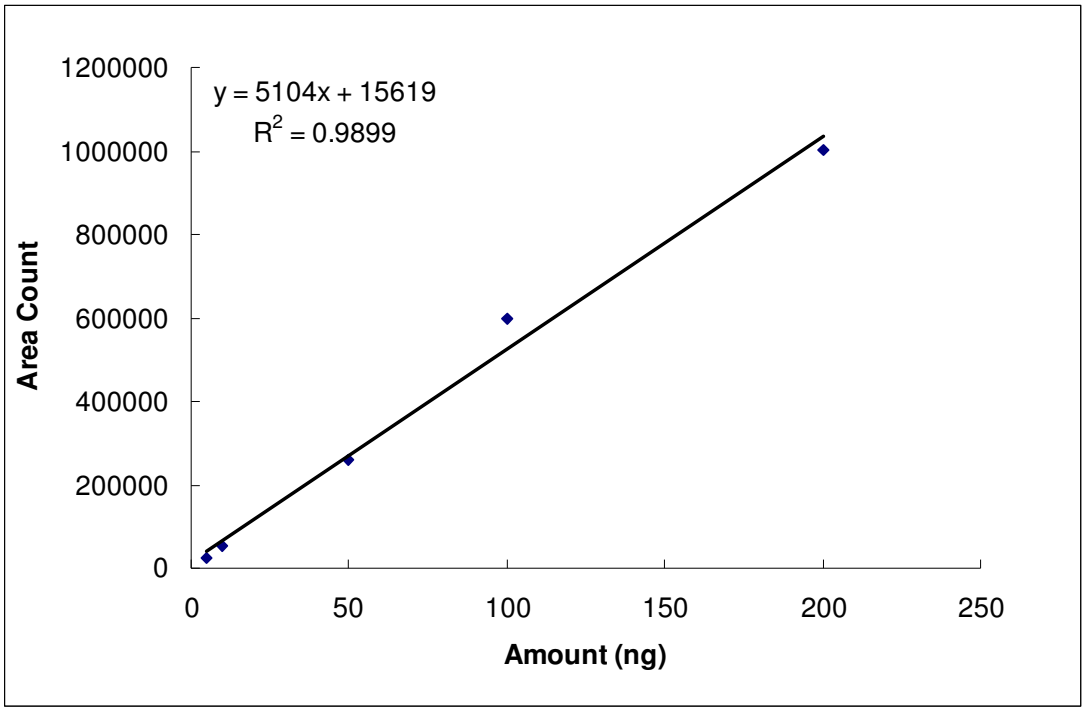

Figure 3.4b External standard curve of ethyl-3-methyl-3-phenylglycidate *trans* isomer (High conc. level)

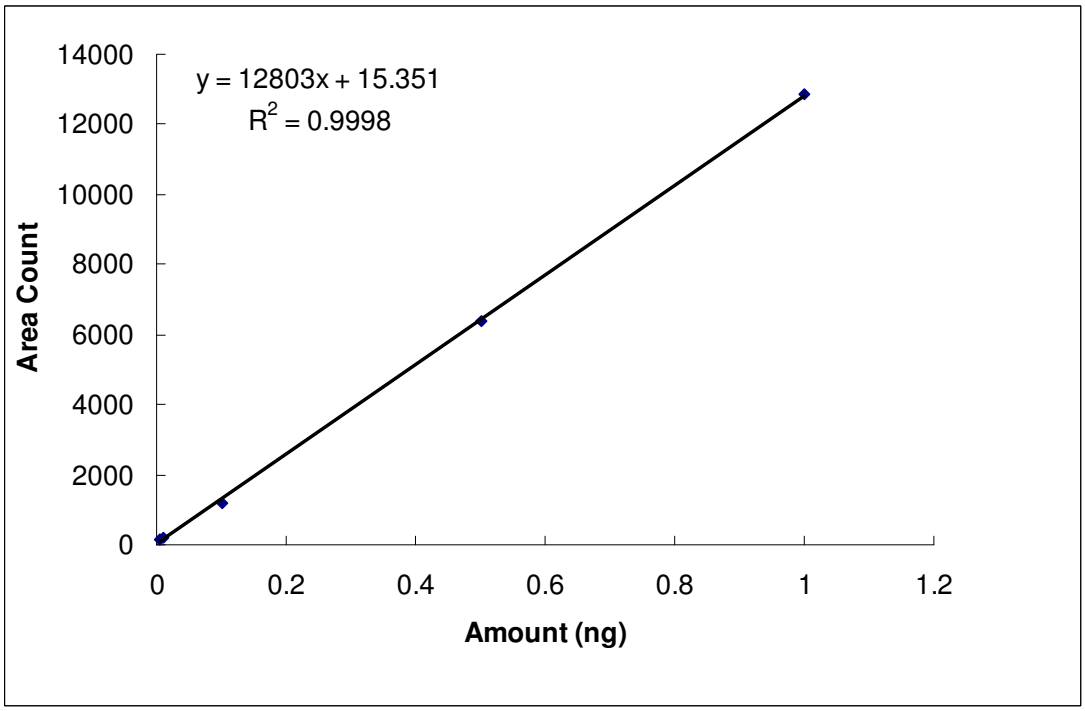

Figure 3.5a External standard curve of γ-undecalactone (Low conc. level)

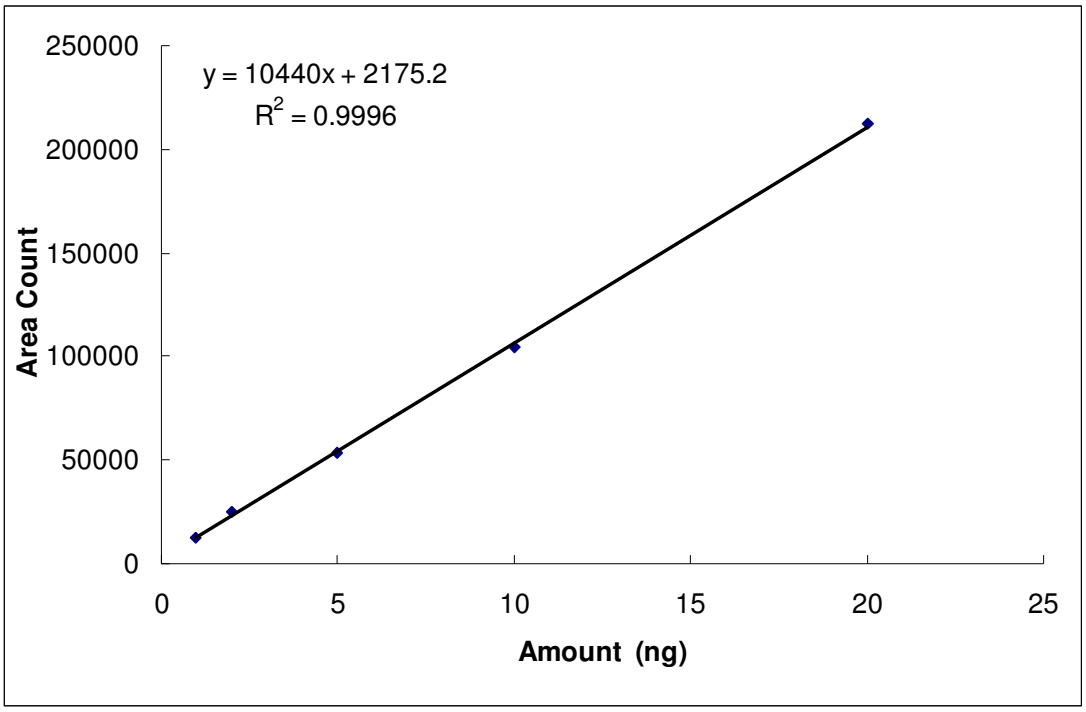

Figure 3.5b External standard curve of γ-undecalactone (High conc. level)

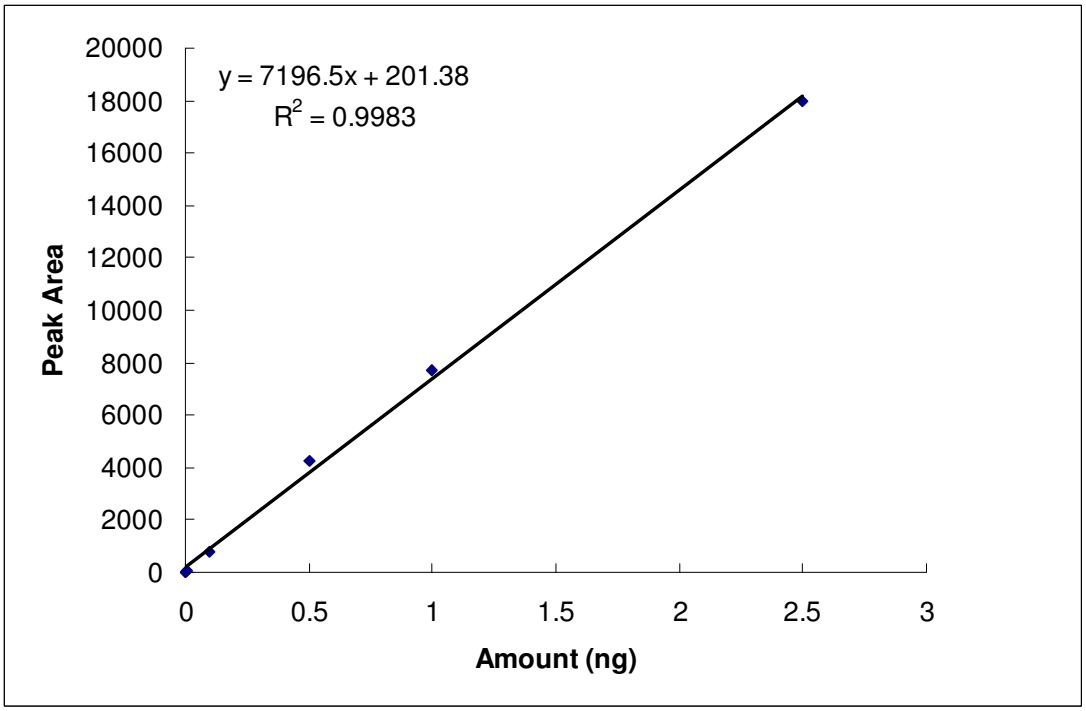

Figure 3.6 External standard curve of Furaneol

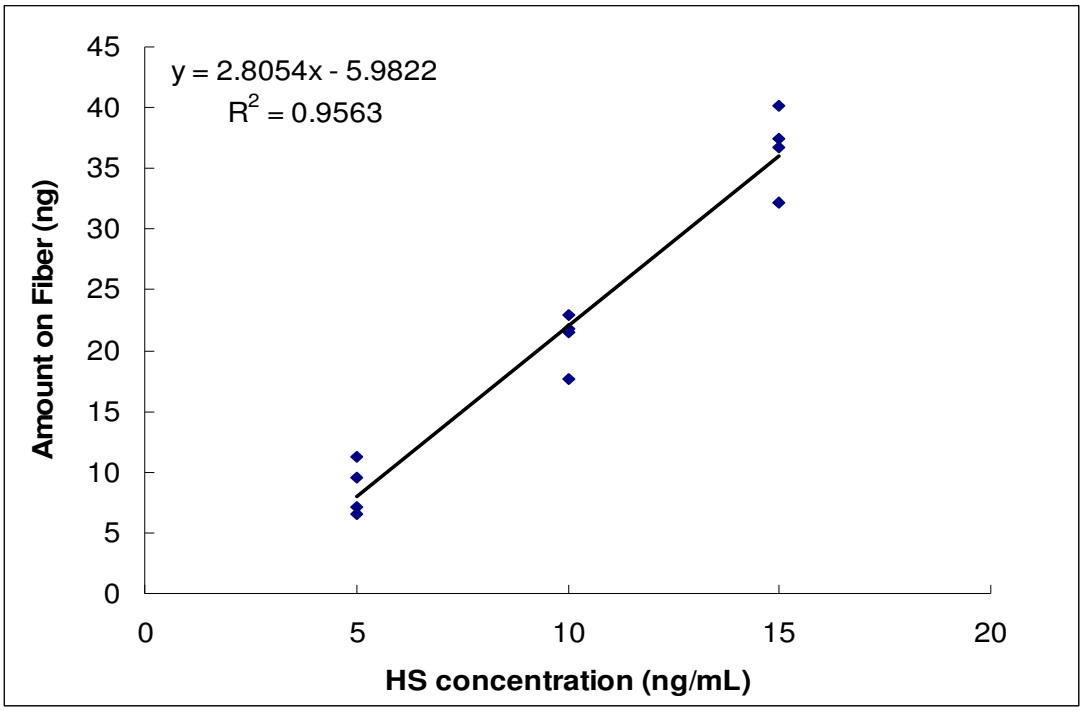

Figure 3.7 Partition coefficient plot of *cis*-3-hexen-1-ol

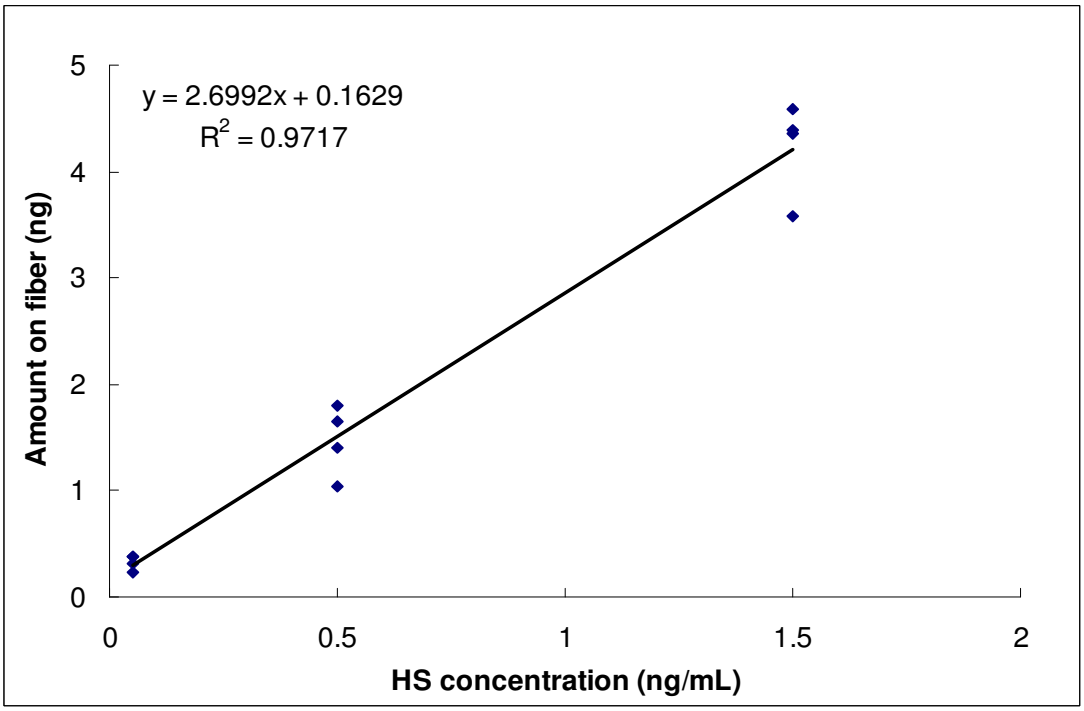

Figure 3.8a Partition coefficient plot of α-ionone (Low conc. level)

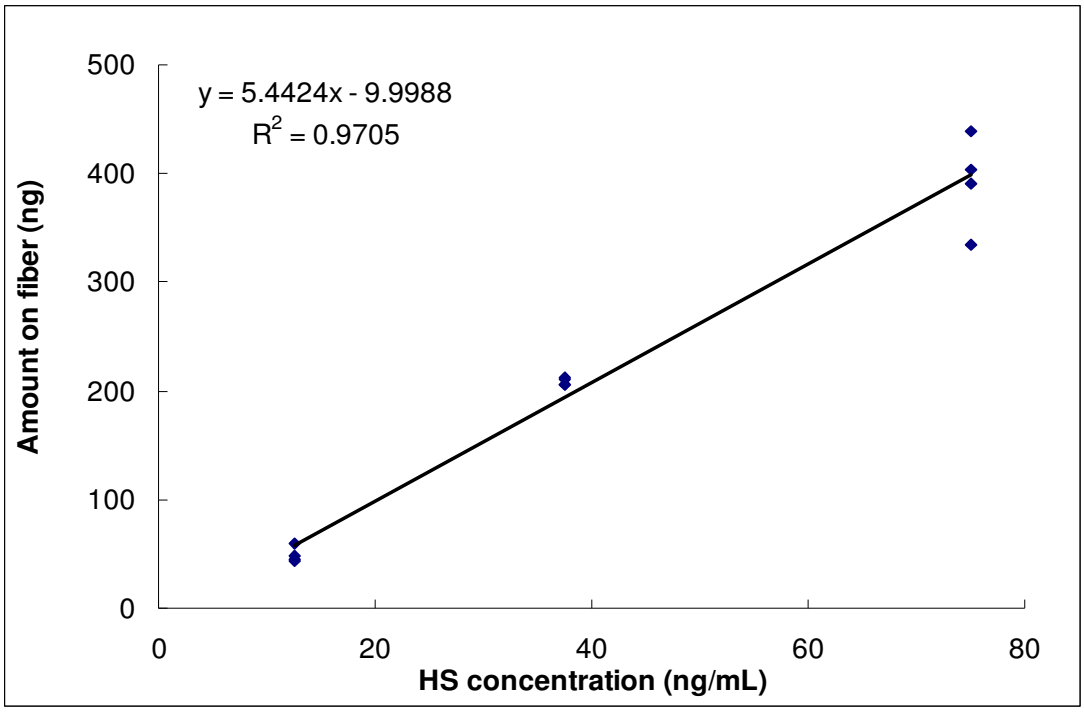

Figure 3.8b Partition coefficient plot of α-ionone (High conc. level)

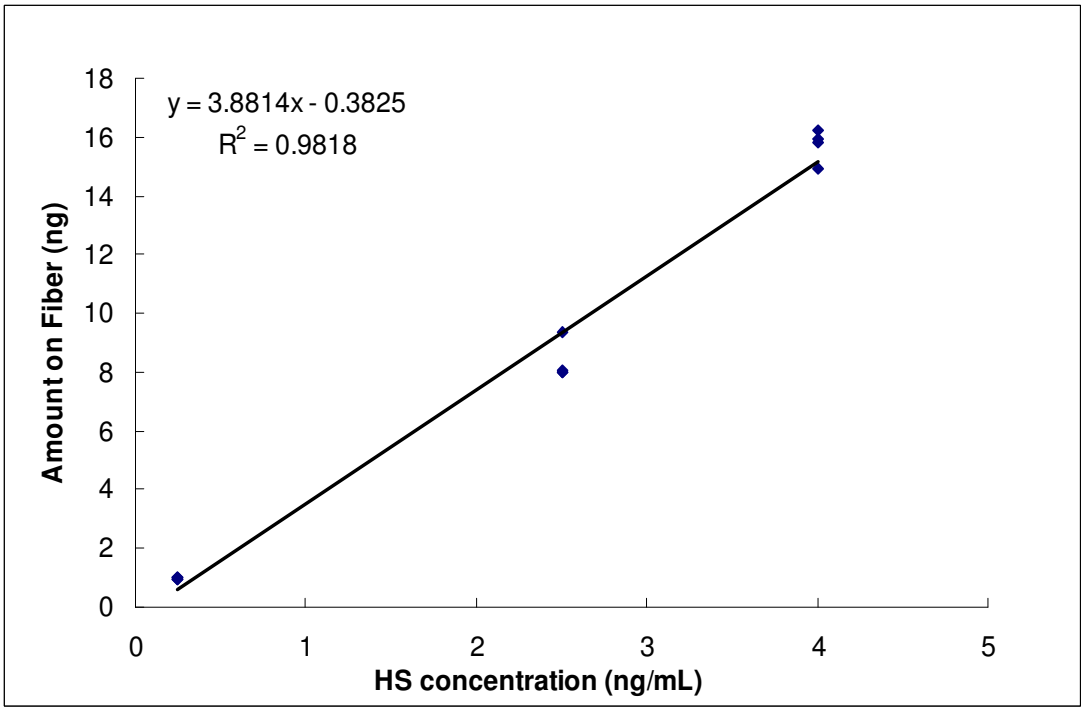

Figure 3.9a Partition coefficient plot of ethyl-3-methyl-3-phenylglycidate *cis* isomer (Low conc. level)

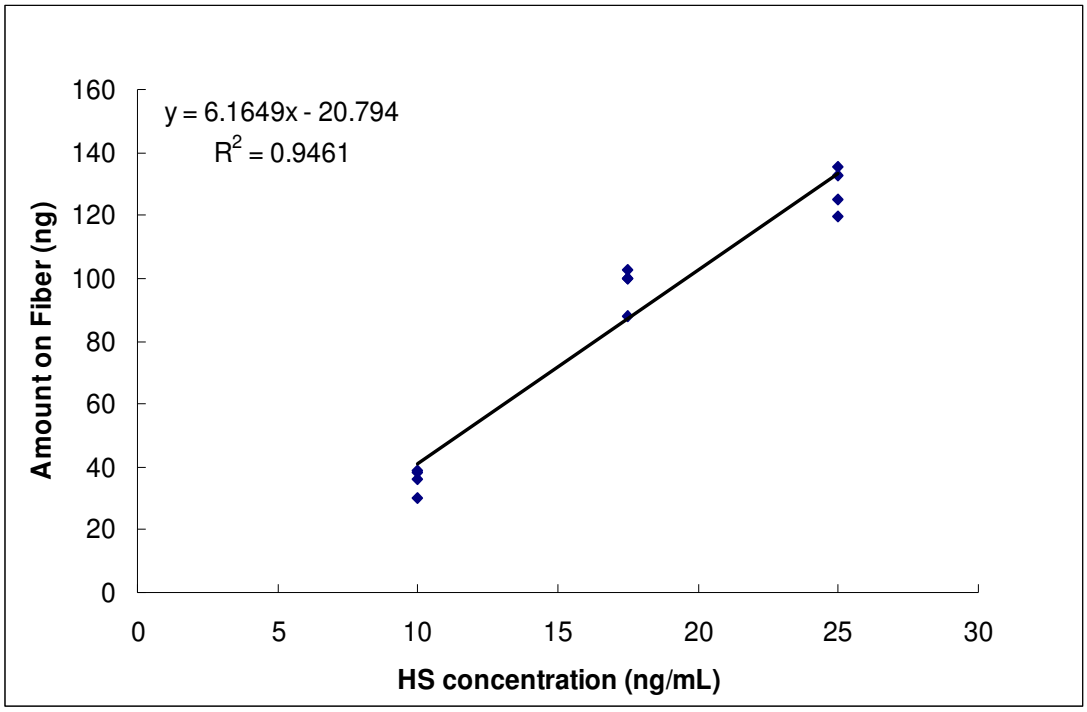

Figure 3.9b Partition coefficient plot of ethyl-3-methyl-3-phenylglycidate *cis* isomer (High conc. level)

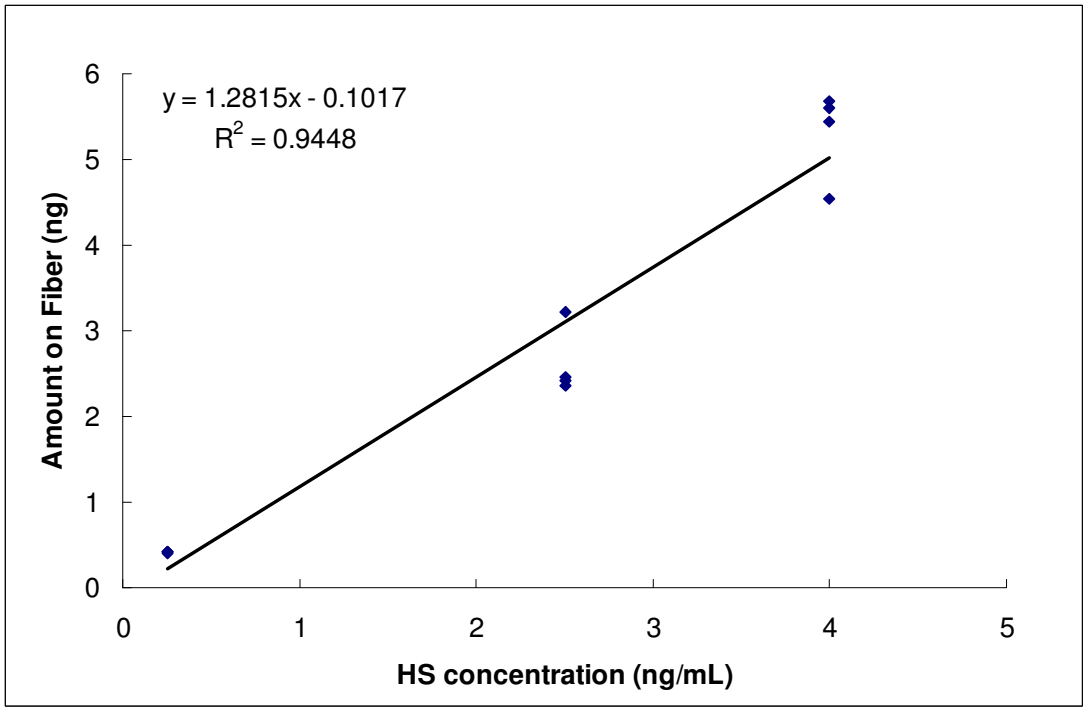

Figure 3.10a Partition coefficient plot of ethyl-3-methyl-3-phenylglycidate *trans* isomer (Low conc. level)

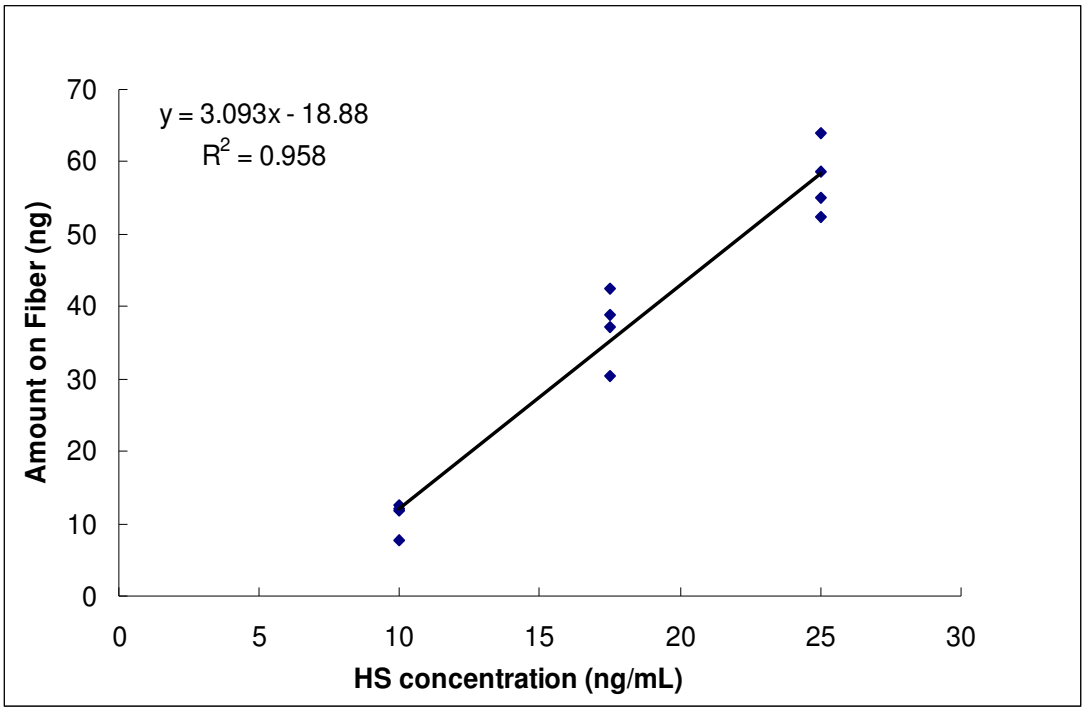

Figure 3.10b Partition coefficient plot of ethyl-3-methyl-3-phenylglycidate *trans* isomer (High conc. level)

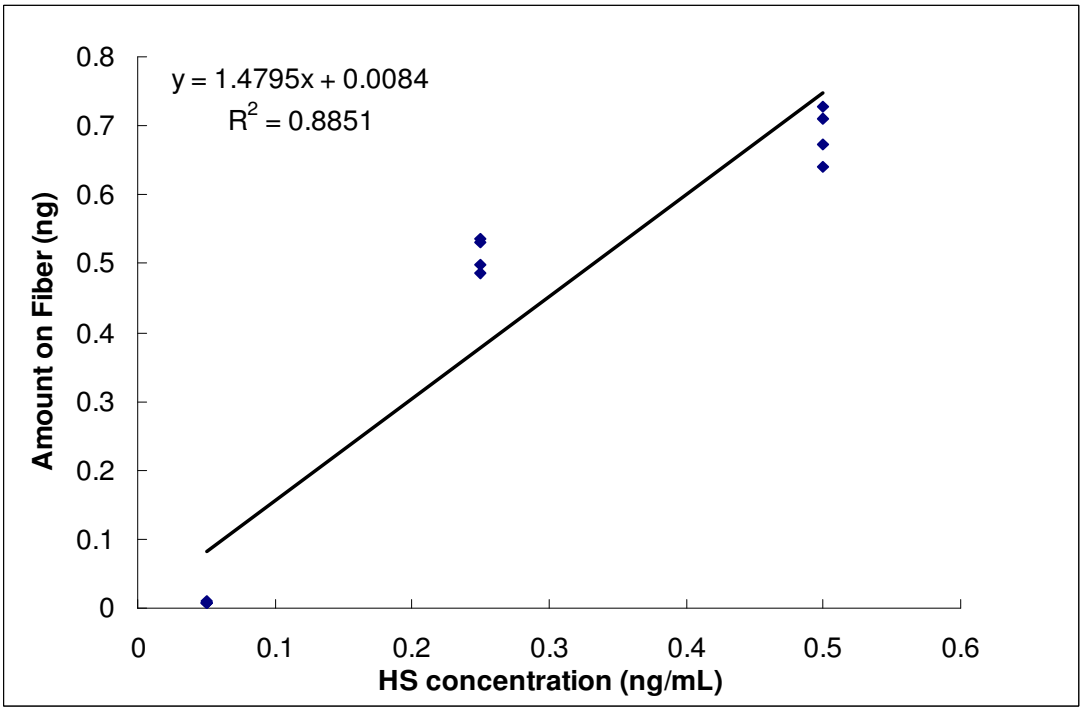

Figure 3.11a Partition coefficient plot of γ-undecalactone (Low conc. level)

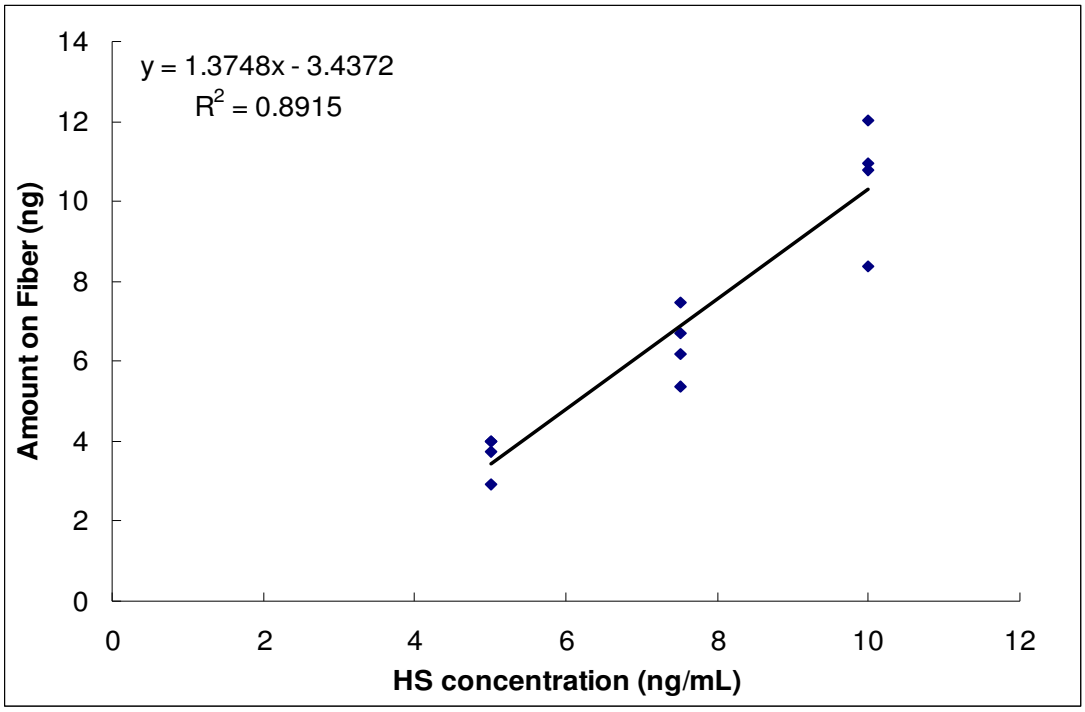

Figure 3.11b Partition coefficient plot of γ-undecalactone (High conc. level)

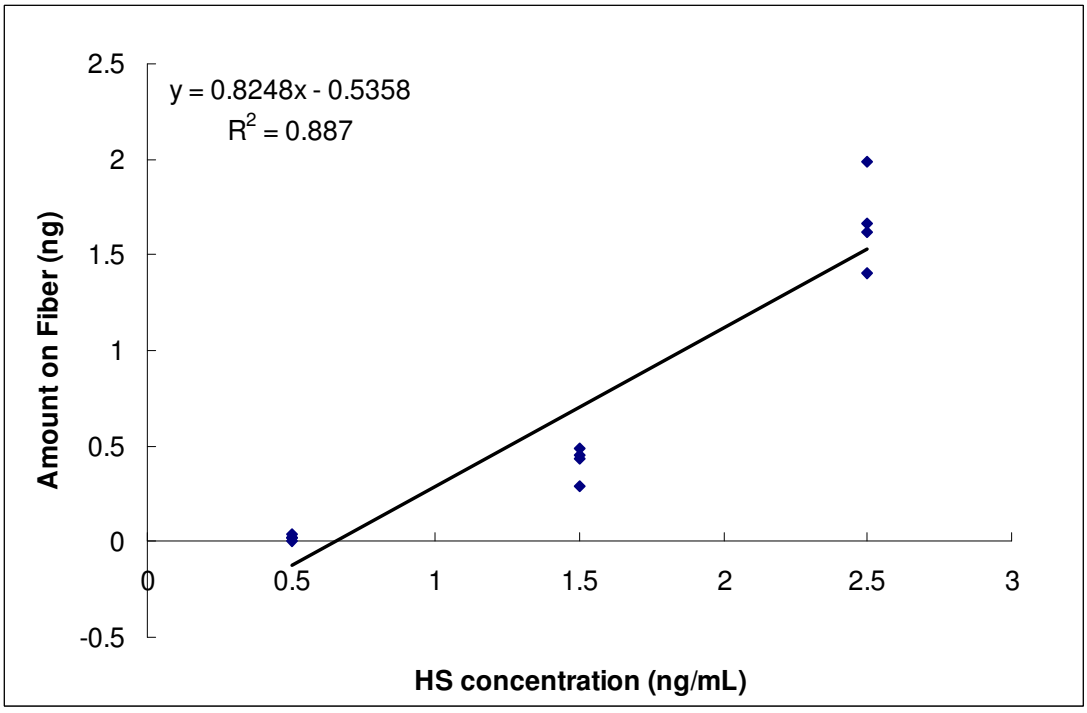

Figure 3.12 Partition coefficient plot of Furaneol

Appendix C Original Data and Calculation of Flavor Released from Emulsion Systems

Table 3.5 Original data and calculation of *cis*-3-hexen-1-ol released from emulsion systems

Fat%	Rep	Area Count	ng on fiber	HS conc.	Total HS amount	Amount released (%)
10	R1	222515	20.4235053	9.41245644	65.88719505	0.585663956
10	R2	151703	13.9696227	7.11193508	49.78354557	0.442520405
10	R3	265912	24.378755	10.8223266	75.75628612	0.67338921
10	R4	256411	23.5128236	10.5136606	73.59562446	0.654183329
4	R1	291566	26.7168884	11.6557669	81.59036826	0.725247718
4	R2	271998	24.9334397	11.0200469	77.14032853	0.685691809
4	R3	262017	24.0237605	10.6957869	74.87050808	0.665515627
4	R4	327262	29.9702607	12.815449	89.7081431	0.797405716
4 +S	R1	293977	26.9366296	11.7340948	82.13866373	0.730121455
4 + S	R2	313502	28.7161593	12.3684178	86.57892465	0.769590441
4 + S	R3	250091	22.9368119	10.3083382	72.15836715	0.641407708
4 + S	R4	310805	28.4703518	12.2807984	85.96558873	0.764138567
4 + L	R1	292800	26.8293565	11.6958568	81.87099729	0.727742198
4 + L	R2	281080	25.761183	11.3151005	79.20570367	0.704050699
4 + L	R3	241731	22.1748724	10.0367407	70.25718501	0.624508311
4 + L	R4	285452	26.1596518	11.4571369	80.19995825	0.712888518
4 + SL	R1	298939	27.3888717	11.8952989	83.26709265	0.740151935
4 + SL	R2	263265	24.1375046	10.7363316	75.15432092	0.668038408
4 + SL	R3	286272	26.2343875	11.4837768	80.38643784	0.714546114
4 + SL	R4	250432	22.967891	10.3194165	72.23591537	0.642097025
0	R1	319918	29.3009205	12.5768591	88.03801371	0.782560122
0	R2	333638	30.5513762	13.0225908	91.1581356	0.810294539
0	R3	291033	26.6683102	11.6384509	81.46915652	0.72417028
0	R4	365254	33.4328928	14.049723	98.34806079	0.874204985

Table 3.6 Original data and calculation of α-ionone released from emulsion systems

Fat%	Rep	Area count	ng on fiber	HS conc.	Total HS amount	Amount released (%)
10	R1	10611	0.827383651	0.246178	1.723245983	0.015317742
10	R2	8585	0.692298973	0.19613181	1.372922648	0.012203757
10	R3	10703	0.833517802	0.24845058	1.739154052	0.015459147
10	R4	8689	0.699233231	0.19870081	1.390905682	0.012363606
4	R1	19295	1.406394186	0.4606899	3.22482932	0.02866515
4	R2	20418	1.481270836	0.48843021	3.419011504	0.030391213
4	R3	28756	2.037211628	0.69439524	4.860766671	0.043206815
4	R4	20290	1.472736365	0.48526836	3.39687854	0.030194476
4 +S	R1	32983	2.319049207	0.79881046	5.591673254	0.049703762
4 + S	R2	23091	1.659494599	0.55445858	3.88121006	0.034499645
4 + S	R3	8415	0.680964129	0.19193247	1.343527304	0.011942465
4 + S	R4	20895	1.513075077	0.50021305	3.501491381	0.031124368
4 + L	R1	19956	1.450466729	0.4770179	3.339125334	0.029681114
4 + L	R2	17468	1.284577944	0.4155594	2.908915829	0.02585703
4 + L	R3	18152	1.330184025	0.43245555	3.02718886	0.026908345
4 + L	R4	18523	1.354920656	0.44161998	3.091339876	0.027478577
4 + SL	R1	21435	1.549079877	0.51355212	3.594864827	0.031954354
4 + SL	R2	18804	1.373656488	0.44856124	3.13992865	0.027910477
4 + SL	R3	21870	1.578083744	0.52429747	3.670082325	0.032622954
4 + SL	R4	22147	1.596552874	0.53113992	3.717979444	0.033048706
0	R1	1751843	181.6487024	35.2137848	246.4964936	2.191079943
0	R2	2276201	238.9693697	45.7460256	320.2221791	2.84641937
0	R3	2198439	230.4687466	44.1841001	309.288701	2.749232897
0	R4	2762622	292.1429196	55.5162648	388.6138537	3.454345366

Table 3.7 Original data and calculation of ethyl-3-methyl-3-phenylglycidate *cis* isomer released from emulsion systems

Fat%	Rep	Area Count	ng on fiber	HS conc.	Total HS amount	Amount released (%)
10	R1	20056	4.84354613	1.34643328	9.425032953	0.083778071
10	R2	13947	3.4181413	0.97919341	6.854353874	0.06092759
10	R3	13849	3.39527509	0.97330218	6.813115284	0.060561025
10	R4	14811	3.61973727	1.03113239	7.217926754	0.064159349
4	R1	42255	10.0232092	2.68091647	18.76641532	0.166812581
4	R2	28648	6.84830603	1.86293761	13.04056326	0.115916118
4	R3	35932	8.54787204	2.30081209	16.10568462	0.143161641
4	R4	29532	7.05456858	1.91607888	13.41255218	0.119222686
4 +S	R1	43143	10.2304051	2.73429821	19.14008745	0.170134111
4 + S	R2	41680	9.88904522	2.6463506	18.5244542	0.164661815
4 + S	R3	29710	7.09610108	1.92677927	13.48745492	0.119888488
4 + S	R4	32514	7.75035466	2.09534051	14.66738358	0.130376743
4 + L	R1	35583	8.46644034	2.27983211	15.95882474	0.14185622
4 + L	R2	35761	8.50797284	2.2905325	16.03372749	0.142522022
4 + L	R3	34239	8.15284661	2.19903813	15.39326693	0.136829039
4 + L	R4	35107	8.35537589	2.25121757	15.75852302	0.14007576
4 + SL	R1	36768	8.74293481	2.35106786	16.45747505	0.146288667
4 + SL	R2	27666	6.61917728	1.80390511	12.62733575	0.112242984
4 + SL	R3	37524	8.91933128	2.39651447	16.77560132	0.149116456
4 + SL	R4	28150	6.73210836	1.83300056	12.83100389	0.114053368
0	R1	268771	66.5282912	14.1644295	99.1510062	0.881342277
0	R2	220592	54.0747538	12.1443582	85.01050732	0.755648954
0	R3	179340	43.4117404	10.4147254	72.90307753	0.648027356
0	R4	241727	59.5378292	13.0305162	91.21361331	0.810787674

Table 3.8 Original data and calculation of ethyl-3-methyl-3-phenylglycidate *trans* isomer released from emulsion systems

Fat%	Rep	Area Count	ng on fiber	HS conc.	Total HS amount	Amount released (%)
10	R1	3727	0.81290398	0.71369799	4.995885962	0.044407875
10	R2	2285	0.57206086	0.52575955	3.680316844	0.032713927
10	R3	2555	0.61715631	0.56094913	3.926643932	0.034903502
10	R4	3153	0.71703439	0.63888755	4.472212818	0.039753003
4	R1	8858	1.66988459	1.38243042	9.677012972	0.086017893
4	R2	6138	1.21558966	1.02792795	7.195495633	0.063959961
4	R3	7215	1.39547041	1.16829529	8.17806702	0.072693929
4	R4	5521	1.11253821	0.94751323	6.632592619	0.058956379
4 +S	R1	9338	1.75005428	1.44498969	10.1149278	0.089910469
4 + S	R2	8923	1.6807409	1.39090199	9.736313938	0.086545013
4 + S	R3	6077	1.20540143	1.01997771	7.139843957	0.06346528
4 + S	R4	6241	1.23279274	1.04135212	7.289464856	0.064795243
4 + L	R1	7323	1.41350859	1.18237112	8.276597856	0.073569759
4 + L	R2	7654	1.46879228	1.22551095	8.57857662	0.076254014
4 + L	R3	6721	1.31296244	1.10391138	7.72737968	0.068687819
4 + L	R4	7441	1.43321698	1.19775027	8.384251917	0.074526684
4 + SL	R1	7512	1.44507541	1.20700383	8.449026818	0.075102461
4 + SL	R2	5619	1.12890618	0.96028575	6.722000229	0.059751113
4 + SL	R3	8014	1.52891955	1.27243039	8.907012739	0.079173447
4 + SL	R4	6071	1.20439931	1.01919572	7.134370022	0.063416622
0	R1	170000	30.2470611	15.8833046	111.1831322	0.988294508
0	R2	135870	23.5601489	13.7213543	96.04948022	0.853773158
0	R3	100351	16.6010972	11.4714184	80.29992895	0.713777146
0	R4	153266	26.9684561	14.8232965	103.7630756	0.92233845

Table 3.9 Original data and calculation of γ-undecalactone released from emulsion systems

Fat%	Rep	Area Count	ng on fiber	HS conc.	Total HS amount	Amount released (%)
10	R1	738	0.05644372	0.03247295	0.227310624	0.001515404
10	R2	237	0.01731227	0.00602384	0.042166877	0.000281113
10	R3	619	0.04714903	0.02619062	0.183334365	0.001222229
10	R4	597	0.04543068	0.02502919	0.1752043	0.001168029
4	R1	990	0.07612661	0.04577669	0.320436821	0.002136245
4	R2	1364	0.10533851	0.06552113	0.458647922	0.003057653
4	R3	1065	0.08198461	0.04973614	0.348152951	0.00232102
4	R4	1334	0.10299531	0.06393735	0.44756147	0.002983743
4 +S	R1	1447	0.11182137	0.06990292	0.489320439	0.003262136
4 + S	R2	2007	0.15556112	0.09946679	0.696267543	0.004641784
4 + S	R3	639	0.04871116	0.02724648	0.190725333	0.001271502
4 + S	R4	1279	0.09869945	0.06103376	0.427236308	0.002848242
4 + L	R1	213	0.01543771	0.00475682	0.033297715	0.000221985
4 + L	R2	850	0.06519167	0.03838572	0.268700045	0.001791334
4 + L	R3	668	0.05097626	0.02877746	0.201442237	0.001342948
4 + L	R4	977	0.07511122	0.04509038	0.315632692	0.002104218
4 + SL	R1	1584	0.12252199	0.07713551	0.53994857	0.003599657
4 + SL	R2	1161	0.08948286	0.05480423	0.383629597	0.002557531
4 + SL	R3	840	0.06441061	0.03785779	0.265004561	0.001766697
4 + SL	R4	1092	0.08409349	0.05116154	0.358130758	0.002387538
0	R1	63137	5.83925287	6.74749263	47.23244844	0.31488299
0	R2	78054	7.26808429	7.78679393	54.50755749	0.363383717
0	R3	58597	5.40438697	6.43118052	45.01826361	0.300121757
0	R4	93667	8.76358238	8.87458712	62.12210985	0.414147399

Table 3.10 Original data and calculation of Furaneol released from emulsion systems

Fat%	Rep	Area Count	ng on fiber	HS conc.	Total HS amount	Amount released (%)
10	R1	3113	0.40458834	1.14014105	7.98098738	0.014188422
10	R2	1967	0.24534426	0.94707113	6.629497881	0.011785774
10	R3	2729	0.35122907	1.07544747	7.52813226	0.013383346
10	R4	1941	0.2417314	0.94269083	6.598835815	0.011731264
4	R1	2359	0.29981519	1.0131125	7.091787483	0.012607622
4	R2	1999	0.24979087	0.95246226	6.667235807	0.011852864
4	R3	1728	0.21213368	0.90680611	6.347642741	0.011284698
4	R4	1248	0.14543459	0.82593912	5.781573841	0.010278353
4 +S	R1	2554	0.32691169	1.04596471	7.321752973	0.01301645
4 + S	R2	2432	0.30995901	1.02541102	7.177877128	0.01276067
4 + S	R3	2027	0.25368165	0.9571795	6.700256493	0.011911567
4 + S	R4	1847	0.22866949	0.92685438	6.487980656	0.011534188
4 + L	R1	2156	0.27160703	0.9789125	6.85238751	0.012182022
4 + L	R2	1883	0.23367192	0.9329194	6.530435823	0.011609664
4 + L	R3	2409	0.30676301	1.02153614	7.150752993	0.01271245
4 + L	R4	858	0.09124158	0.76023469	5.321642859	0.009460698
4 + SL	R1	2598	0.33302578	1.05337752	7.373642622	0.013108698
4 + SL	R2	2245	0.28397415	0.99390659	6.957346119	0.012368615
4 + SL	R3	1687	0.20643646	0.89989872	6.299291022	0.01119874
4 + SL	R4	789	0.08165358	0.74861006	5.240270455	0.009316036
0	R1	1810	0.2235281	0.92062088	6.444346178	0.011456615
0	R2	1251	0.14585146	0.82644454	5.785111771	0.010284643
0	R3	1240	0.14432293	0.82459134	5.772139359	0.010261581
0	R4	801	0.08332106	0.75063174	5.254422177	0.009341195

Appendix D SAS Programs

SAS Program 1: The analyses of variance (ANOVA) for determine significant differences between K value and the amount of flavor compound in the headspace (Partition Coefficient).

```
options pagesize=60 linesize=72;
data fu;
input amount$ rep$ kvalue;
cards;
;
proc anova;
class amount rep;
model kvalue=amount rep;
means amount/lsd;
run;
```

SAS Program 2: The General Linear Model (GLM) procedure for the determination of flavor released from different fat treatments.

```
data compound;
input product$ rep$ CH AI EMP1 EMP2 GU FU;
lch=log10(ch);
lai=log10(ai);
lemp1=log10(emp1);
lemp2=log10(emp2);
lgu=log10(gu);
lfu=log10(fu);
cards;
;
proc print;
proc glm ;
class product rep;
model CH AI EMP1 EMP2 GU FU lch lai lemp1 lemp2 lgu lfu=product rep;
means product rep/lsd;
means product ;
run;
```

The GLM Procedure for *cis*-3-hexen-1-ol

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.10984720	0.01373090	3.45	0.0187
Error	15	0.05975594	0.00398373		
Corrected Total	23	0.16960314			

R-Square	Coeff Var	Root MSE	CH Mean
0.647672	8.984528	0.063117	0.702505

Source	DF	Type I SS	Mean Square	F Value	Pr > F
product	5	0.09213272	0.01842654	4.63	0.0094
rep	3	0.01771448	0.00590483	1.48	0.2595

Source	DF	Type III SS	Mean Square	F Value	Pr > F
product	5	0.09213272	0.01842654	4.63	0.0094
rep	3	0.01771448	0.00590483	1.48	0.2595

The GLM Procedure forα-ionone

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	14.20387167	1.77548396	106.18	<.0001
Error	15	0.25081585	0.01672106		
Corrected Total	23	14.45468751			

R-Square	Coeff Var	Root MSE	lai Mean
0.982648	-10.31812	0.129310	-1.253231

Source	DF	Type I SS	Mean Square	F Value	Pr > F
product	5	14.19058239	2.83811648	169.73	<.0001
rep	3	0.01328928	0.00442976	0.26	0.8496

Source	DF	Type III SS	Mean Square	F Value	Pr > F
product	5	14.19058239	2.83811648	169.73	<.0001
rep	3	0.01328928	0.00442976	0.26	0.8496

The GLM Procedure for ethyl-3-methyl-3-phenylglycidate *cis* isomer

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1.43406584	0.17925823	110.39	<.0001
Error	15	0.02435752	0.00162383		
Corrected Total	23	1.45842336			

R-Square	Coeff Var	Root MSE	EMP1 Mean
0.983299	17.33697	0.040297	0.232433

Source	DF	Type I SS	Mean Square	F Value	Pr > F
product	5	1.42421374	0.28484275	175.41	<.0001
rep	3	0.00985210	0.00328403	2.02	0.1540

Source	DF	Type III SS	Mean Square	F Value	Pr > F
product	5	1.42421374	0.28484275	175.41	<.0001
rep	3	0.00985210	0.00328403	2.02	0.1540

The GLM Procedure for ethyl-3-methyl-3-phenylglycidate *trans* isomer

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	4.58502177	0.57312772	204.98	<.0001
Error	15	0.04193944	0.00279596		
Corrected Total	23	4.62696120			

R-Square	Coeff Var	Root MSE	lemp2 Mean
0.990936	-5.230522	0.052877	-1.010929

Source	DF	Type I SS	Mean Square	F Value	Pr > F
product	5	4.55930849	0.91186170	326.14	<.0001
rep	3	0.02571328	0.00857109	3.07	0.0603

Source	DF	Type III SS	Mean Square	F Value	Pr > F
product	5	4.55930849	0.91186170	326.14	<.0001
rep	3	0.02571328	0.00857109	3.07	0.0603

The GLM Procedure for γ-undecalactone

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	18.60736995	2.32592124	31.56	<.0001
Error	15	1.10547731	0.07369849		
Corrected Total	23	19.71284726			

R-Square	Coeff Var	Root MSE	lgu Mean
0.943921	-11.42478	0.271475	-2.376192

Source	DF	Type I SS	Mean Square	F Value	Pr > F
product	5	18.51635861	3.70327172	50.25	<.0001
rep	3	0.09101134	0.03033711	0.41	0.7470

Source	DF	Type III SS	Mean Square	F Value	Pr > F
product	5	18.51635861	3.70327172	50.25	<.0001
rep	3	0.09101134	0.03033711	0.41	0.7470

The GLM Procedure for 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.00003286	0.00000411	9.58	0.0001
Error	15	0.00000643	0.00000043		
Corrected Total	23	0.00003929			

R-Square	Coeff Var	Root MSE	FU Mean
0.836373	5.618795	0.000655	0.011652

Source	DF	Type I SS	Mean Square	F Value	Pr > F
product	5	0.00001394	0.00000279	6.50	0.0021
rep	3	0.00001892	0.00000631	14.72	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
product	5	0.00001394	0.00000279	6.50	0.0021
rep	3	0.00001892	0.00000631	14.72	<.0001

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