

## ABSTRACT

KANYUCK, KELSEY M. Weighting Agent and Flavor Compound Interactions: the Impacts of Concentration, Chemistry, and Oil Composition for Application in Beverage Emulsions (Under the direction of Dr. Christopher Daubert).

Widely used in beverages, such as lemon-lime sodas and orange sports drinks, beverage weighting agents (WAs) are added to flavor oils to increase stability of the emulsion. Flavor oils have a low density, and the high density WAs increase the overall oil phase density, near the aqueous phase density, to inhibit gravitational separation. Common WAs in the United States are sucrose acetate isobutyrate (SAIB), brominated vegetable oil (BVO), and ester gum (EG). Due to consumer demands for 'clean' ingredients, companies have begun assessing the replacement of traditional WAs for newer WAs. Ingredient substitutions commonly alter properties of a product, and the effects of replacing WAs on flavor has not been previously addressed. This thesis addresses the impacts of WA chemistry and concentration on volatile components of flavor oils. With the addition of WAs or chemical analogs, changes in flavor compound volatility were measured with headspace gas chromatograph mass spectrometry (GC-MS). A greater concentration of WA corresponded to a greater decrease in flavor compound availability (indicating a smaller RHI), although individual molecules responded differently. The chemistry and concentration of flavor molecules affected the magnitude of changes in volatility with WA addition. Multivariate clustering and modeling was used to assess the importance of molecule chemistry in the association strength to WAs with each flavor compound, and oxygenated compounds with the greatest polarity and polarizability (such as citronellal and citral) had the strongest associations with WAs. Terpenoid molecules had the strongest association to either WA (centroid RHI of 46% with SAIB at 0.52 m) and terpene molecules displayed the lowest

association (centroid RHI of 91% with SAIB at 0.52 m). The chemistry of SAIB and BVO was also suggested to impact flavor compound associations. A sucrose backbone resulted in larger decreases in compound volatility than a glycerol backbone ( $K_f$  of octanal 0.35 vs 0.27), and addition of a brominated oil decreased compound volatility more than non-brominated oil ( $K_f$  of octanal 0.35 vs 0.24). The model of predicting flavor compound interactions and the method for assessing WA association strength provide tools for beverage manufacturers to predict the effect of substituting WAs and insight into molecular influences of WAs for designing a new WA.

© Copyright 2017 Kelsey M. Kanyuck

All Rights Reserved

Weighting agent and flavor compound interactions: The impacts of concentration, chemistry,  
and oil composition for application in beverage emulsions

by  
Kelsey M. Kanyuck

A thesis submitted to the Graduate Faculty of  
North Carolina State University  
in partial fulfillment of the  
requirements for the degree of  
Masters of Science

Food Science

Raleigh, North Carolina

2017

APPROVED BY:

---

Dr. Christopher Daubert  
Committee Chair

---

Dr. MaryAnne Drake

---

Dr. Suzanne Johanningsmeier

---

Dr. Jason Osborne  
Minor Member

## **BIOGRAPHY**

Kelsey Kanyuck grew up in Prince George's County, Maryland with parents Bob and Mary Ellen and brother Alec. She attended the University of Maryland, College Park and earned a B.S. in Chemistry and a B.S. in Food Science in 2015. After graduation, she moved to Raleigh, NC to begin pursuing a master's degree at North Carolina State University. While at NC State, she has been an active member of the food science club and the IFTSA board of directors. Kelsey has held internships at Mondelez International, The Hershey Company, the FDA, and the USDA. After graduation, Kelsey plans to pursue a PhD at the University of Birmingham in the U.K.

## ACKNOWLEDGMENTS

I would like to thank everyone who has supported and taught me throughout my journey the past two years. Dr. Christopher Daubert, thank you for your guidance and support that encouraged me grow academically, professionally, and personally. Your belief that a master's program is more than just a thesis, but an opportunity to take advantage of all that a university offers (including golf lessons), allowed me to develop tremendously more than I could have imagined. Chris Pernell, thank you for your fruitful discussion and teamwork throughout every stage of this project and teaching me the nuances of 'southern' culture. The two of you make a great duo, and I was honored to learn from you both. Thank you to my committee members, Dr. MaryAnne Drake, Dr. Suzanne Johanningsmeier, and Dr. Jason Osborne, for your insightful suggestions and discussion. I would also like to thank Eastman Chemical Company for the financial support and, more personally, Andy Singleton, Barclay Satterfield, Ignacio Garcia, Jim Ponasik, and Weimin Liang for their guidance and suggestions. To my parents, thank you for always supporting me and teaching me to follow my dreams. To Erin, Hannah, Sarah, Meg, Andy, Kyle, Bryan, and Kristen, thank you for your friendship and joining me on the roller coaster that is graduate school.

## TABLE OF CONTENTS

LIST OF TABLES .....	vii
LIST OF FIGURES .....	viii
CHAPTER 1: INTRODUCTION .....	1
CHAPTER 2: LITERATURE REVIEW .....	4
<b>2.1. Introduction</b> .....	4
2.1.1 Overview of Emulsions in Soft Drinks .....	4
2.1.2 Objectives and Motivation .....	4
<b>2.2. Beverage Flavor Emulsions Overview</b> .....	5
2.2.1 Composition and Usage Levels .....	5
2.2.2 Approaches to Improve Emulsion Stability .....	6
2.2.2.1 Weighting Agents .....	7
2.2.2.2 Emulsifiers .....	11
2.2.2.3 Homogenization .....	12
<b>2.3. Citrus Flavor Oil Composition</b> .....	12
2.3.1 Orange Oil .....	14
2.3.2 Lemon Oil .....	15
2.3.3 Lime Oil .....	16
<b>2.4. Molecular Interactions</b> .....	16
2.4.1 Partitioning Coefficients .....	16
2.4.2 Formation Constants ( $K_f$ ) .....	19
2.4.3 Statistical Modeling By Molecular Properties (QSAR) .....	20
2.4.4 Statistical Clustering .....	22
<b>2.5. Flavor Changes Observed with Sample Modifications</b> .....	22
2.5.1 Ingredient Modifications .....	23
2.5.1.1 Oil Phase Interactions .....	23
2.5.1.2 Beverage Additive Interactions .....	24
2.5.2 Processing and Droplet Size Modifications .....	26
<b>2.6. Flavor Perception Measurements</b> .....	27
2.6.1 Overview of Aroma and Taste Perception .....	27
2.6.2 Headspace Gas Chromatography with Mass Spectrometry .....	28

2.6.3 Trained Panel Sensory Analysis .....	29
2.6.4 Consumer Sensory Difference Testing .....	29
<b>2.7. Conclusion</b> .....	30
<b>2.8. References</b> .....	31
CHAPTER 3: INTERACTIONS BETWEEN WEIGHTING AGENTS AND FLAVOR COMPOUNDS IN ESSENTIAL CITRUS OIL BLENDS .....	46
<b>3.1. Abstract</b> .....	46
<b>3.2. Introduction</b> .....	47
3.2.1. Background .....	47
3.2.2. Measurement of Flavor-Ingredient Interactions .....	48
3.2.3. Research Objectives.....	50
<b>3.3. Materials and Methods</b> .....	51
3.3.1. Sample Preparation .....	51
3.3.2. Instrumental Analysis .....	52
3.3.3. Volatile Compounds Calculations .....	53
3.3.4. Data Analysis and Modeling.....	53
<b>3.4. Results and Discussion</b> .....	54
3.4.1. Physical Properties of Flavor Blends .....	54
3.4.2. Implications of WA Concentration.....	55
3.4.3. Influences of Flavor Compound Structures .....	57
3.4.4. Effects of Flavor Oil Composition.....	59
3.4.5. Predicting the Influences of Flavor Compound Structures.....	60
3.4.6. Adjustments to Account for WA Differences.....	63
<b>3.5. Conclusion</b> .....	64
<b>3.6. References</b> .....	65
CHAPTER 4: MOLECULAR INFLUENCES OF WEIGHTING AGENTS AND THE IMPLICATIONS ON COMPLEXATION WITH ORANGE OIL AROMA COMPOUNDS .....	78
<b>4.1. Abstract</b> .....	78
<b>4.2. Introduction</b> .....	79
<b>4.3. Materials and Methods</b> .....	83
4.3.1 Flavor Oil Blends.....	83
4.3.2 Volatility Measurements.....	84

<b>4.4. Results and Discussion</b> .....	85
4.4.1. Importance of Additive Concentration .....	85
4.4.2. Comparison of Weighting Agents .....	86
4.4.3. Influences of Flavor Molecule Chemistry .....	87
4.4.4. Molecular Structure Importance Through Analog Comparison .....	89
<b>4.5. Conclusion</b> .....	94
<b>4.6. References</b> .....	95
CHAPTER 5: CONCLUSION .....	109
APPENDIX A: WEIGHTING AGENT EFFECTS ON FLAVOR OIL SOLUBILITY AND VOLATILITY IN MODEL SYSTEMS AND BEVERAGES .....	112
<b>A.1. Abstract</b> .....	112
<b>A.2. Introduction</b> .....	112
<b>A.3. Methods and Materials</b> .....	114
A.3.1. Sample Preparation .....	114
A.3.2. UV Spectrophotometry .....	115
A.3.3. Gas Chromatography Mass Spectrometry .....	115
A.3.4. Triangle Tests.....	116
A.3.5. Descriptive Analysis .....	116
<b>A.4. Results and Discussion</b> .....	117
A.4.1. Measurement of Solubility with Model Systems.....	117
A.4.2. Sensory Analysis of Beverage Systems.....	119
<b>A.5. Conclusions</b> .....	120
<b>A.6. References</b> .....	121
APPENDIX B: SUPPLEMENTAL MATERIAL .....	129

## LIST OF TABLES

Table 2.1. Common weighting agents and information relevant to their usage in commercial beverages.....	39
Table 2.2. Typical soda formulation with a concentrated flavor emulsion (by weight percentages). .....	40
Table 2.3. Flavor Activity of Volatile Compounds Present in Orange Essential Oils.....	41
Table 2.4. Chemical properties of key flavor compounds in citrus essential oils including the octanal:water partition coefficient (logP) and Molar Refractivity (A) in (cm <sup>3</sup> ). .....	42
Table 3.1. Weighting agent properties, and qualities within orange oil blends.....	74
Table 3.2. Orange oil volatile compounds measured for each of the WAs at usage ratios with a comparison to the molecular properties logP and A (molar refractivity). .....	75
Table 3.3. Clusters RHI for each of the WAs at usage ratios in orange oil with a comparison of the molecular properties. ....	76
Table 3.4. List of Abbreviations .....	77
Table 4.1. Experimental design variables and ingredient comparisons used to contrast each variable.....	98
Table 4.2. Orange oil volatile compounds associations with chemical analogs as measured by K <sub>f</sub> (m <sup>-1</sup> ) for each individual compound.....	99
Table 4.3. Comparison of K <sub>f</sub> for each flavor compound cluster with each of the WA chemical analogs. ....	100
Table 4.4. Molecular characterization of assumed pure compound in each chemical analog with molecular properties .....	101
Table 4.5. List of Abbreviations .....	102
Table A.1. Comparison of water soluble components with the inclusion of WAs into flavor oils in a static system .....	125
Table A.2. Triangle tests statistics for comparisons of SAIB, BVO, and EG with orange and lemon flavor oils .....	126
Table A.3. Overall p-values for comparisons of each measured attribute between essential oil model beverages with SAIB and BVO.for 7 people and 4 replicates each (2 batch reps and 2 panel reps).....	127
Table A.4. Percent of total peak area for volatile compounds in headspace of beverages flavored with orange and lemon oils.....	128
Table B.1. Volatile compounds RHI (%) with inclusion of SAIB, BVO, and EG at concentrations of 0.52, 0.28, 0.15, and 0.04 molality in orange oil flavor blends.....	129
Table B.2. Molecular properties for each volatile compound utilized as possible parameters in model selection. ....	130
Table B.3. Correlation (R <sup>2</sup> ) of the chosen variables with the predicted value (K <sub>f</sub> ) and each non-transformed variable utilized as a possible parameter in model selection. ....	131

## LIST OF FIGURES

Figure 2.1. Mechanisms of physical instability in emulsions .....	43
Figure 2.2. Chemical structures of the three most common weighting agents .....	44
Figure 2.3. Schematic illustration of a beverage emulsion .....	45
Figure 3.1. Impacts of weighting agent molality on the volatility of two selected compounds in orange oil (A: linalool, an alcohol; B: $\beta$ -pinene, a terpene). Error bars represent one standard deviation of each data point, which were run in triplicate. ....	68
Figure 3.2. Variability in magnitudes of orange oil volatile compounds interactions with SAIB and BVO .....	69
Figure 3.3. Variability in magnitudes of orange oil volatile compounds interactions with EG and BVO at typical usage ratios.....	70
Figure 3.4. Comparison of the magnitude of (A) orange, (B) lemon, and (C) lime flavor oil components volatility changes .....	71
Figure 3.5. Quality of the linear model $K_f$ .....	72
Figure 3.6. Volatility profiles and impacts of spiking within orange oil blends .....	73
Figure 4.1. Comparison of the molecular structures of WAs SAIB and BVO and the chemical analogs utilized in this experiment Gly C <sub>4</sub> , Gly C <sub>8</sub> , Gly C <sub>18</sub> . ....	103
Figure 4.2. Volatility changes with increasing analog concentrations in orange oil for flavor compounds A: linalool and B: $\beta$ -pinene. Error bars represent one standard deviation of six replicates .....	104
Figure 4.3. Comparison of the complexation of flavor compounds in orange oil to WAs SAIB and BVO for (A) each flavor compounds and (B) clusters of flavor compounds .....	105
Figure 4.4. Effects of molecule backbone with a comparison of sucrose plotted against glycerol both with C <sub>4</sub> chain length.....	106
Figure 4.5. Effects of molecule chain length with a comparison of C <sub>4</sub> plotted against C <sub>18</sub> both with glycerol backbones. ....	107
Figure 4.6. Effects of molecule bromination with a comparison of brominated vegetable oil plotted against C <sub>18</sub> non-brominated oil. ....	108
Figure A.1. Comparison of the solubility of chromophore components within orange, lemon, and lime flavor oils over the UV spectrum. ....	123
Figure A.2. Impacts of WAs on the solubility kinetics of orange oil components into an aqueous phase .....	124

## CHAPTER 1: INTRODUCTION

Weighting agents (WAs) are often added to citrus flavor oils in beverages, such as sodas and sports drinks, to increase the density of the oil phase to increase emulsion stability. Without the inclusion of a high-density WA, the low-density oil phase would rise and separate from the aqueous phase, in a process called creaming (or ringing in beverages). Several WAs are commercially used, including sucrose acetate isobutyrate (SAIB), ester gum (EG), and brominated vegetable oil (BVO). Beverage companies have begun switching from BVO to alternative WAs, due to consumer complaints. Sensorial changes have been observed when switching between other emulsion ingredients, but the impacts of substituting WAs on flavor compounds has not been established.

The goal of this work was to establish a fundamental understanding of the influences of WAs in beverage flavor emulsions. Within the oil phase, molecular interactions between flavor compounds and the WAs were hypothesized to impact flavor availability and migration. The structure and chemistry of both the flavor compound and the WA were likely important factors in the interaction, and thus both were analyzed in this thesis. It was further hypothesized that emulsion properties and processing conditions can contribute to the flavor release of the model beverage systems. Towards this goal, three hypotheses were proposed and addressed in this thesis:

Hypothesis A: Intermolecular forces from molecular, physiochemical interactions between a WA and flavor molecules selectively modifies the ratio of free flavor compounds and is based on the flavor molecule chemistry. The objective was to characterize the type and extent of changes in flavor compounds with the inclusion of WAs. For this objective, the impacts of WA type and concentration were analyzed in three citrus essential oils. Changes

in volatility of individual flavor compounds was analyzed and compared for each WA at concentrations spanning permitted usage ratios. Multivariate clustering and linear regression was utilized to relate the functional groups of volatile molecules to their interactions with WAs. These objectives and research premise is further detailed in Chapter 3.

Hypothesis B: Molecular differences between SAIB and BVO cause differences in flavor interactions, based on the underlying chemical structures. To address this hypothesis, the objective was to analyze each of the chemical differences between the WAs and the contribution of these differences to flavor compound interactions. Towards this objective, chemical analogs were used to compare the effects of the major molecular differences between SAIB and BVO : backbone, chain length, and bromination. Connections were made between analog functional groups and degree of flavor compound changes. Underlying chemical interactions between flavor compounds and WAs were proposed, based on the influence of each molecular structures. These objectives and research premise is further detailed in Chapter 4.

Hypothesis C: The miscibility of flavor molecules into an aqueous phase is modified by different WAs and homogenization parameters for the emulsification of flavor oil systems. The objective of this part was to describe differences between model beverages when different processing or weighting agents were utilized. Homogenization pressure and emulsifier concentration were related to the resulting droplet size of the emulsion and compared to the volatile headspace concentration of flavor compounds. Sensory analysis with consumer testing determined whether a perceivable difference existed between beverages with different WAs. Trained panel measurements qualitatively described

differences in taste or flavor of these beverages. The work done towards addressing this hypothesis is presented and further explained in Appendix 1.

This thesis delivers the first published work on characterization of WA and flavor compound interactions and provides analysis of the influences of the molecular chemistry of these ingredients. Further, generalized models were proposed for predicting the effects of new WAs or flavor compounds. Previous studies regarding beverage emulsions have focused on the impacts of emulsifier on flavor compounds (Mirhosseini and Tan, 2009; Mirhosseini et al. 2008) or the impacts of WAs on stability (Chanamai and McClements, 2000; Lim et al. 2011). Investigation into the impacts of the inclusion of WAs on flavor compounds will assist beverage manufacturers in reformulating products with a new WA as they address consumer demands for new ingredients.

## CHAPTER 2: LITERATURE REVIEW

### 2.1. Introduction

#### 2.1.1 Overview of Emulsions in Soft Drinks

Around the world, the most popular form of non-alcoholic beverages are orange flavored drinks, including sodas and flavored waters, which utilize essential oil flavoring (Tan, 2004). These essential oils are immiscible in water, and therefore an emulsion is formed in most citrus flavored beverages. Like all emulsions, the mixing of immiscible phases is not stable and requires additional ingredients to prevent separation over time. Towards this goal, weighting agents (WAs) inhibit gravitational separation, while emulsifiers decrease the merging of oil droplets (McClements, 2016). Recent changes in consumer perception have motivated beverage manufacturers to modify formulations away from conventional WAs, and these types of ingredient switches may impact the flavor of the product.

#### 2.1.2 Objectives and Motivation

Brominated vegetable oil (BVO) has historically been used throughout the food industry as a WA. Recent awareness has changed public opinion about the safety of bromine, motivating beverage manufacturers to seek alternative ingredients (in Europe, BVO is no longer permitted for use (Tan, 2004)). The two most widely used alternative weighting agents are sucrose acetate isobutyrate (SAIB) and glycerol ester of wood rosin, also known as ester gum (EG). As of 2017, all three of these ingredients are permitted for use in the United States (Table 2.1). Other ingredient changes have been reported to inadvertently change the perceived flavor of the original product (such as reduced fat formulations) (Hatchwell, 1996; Deibler and Acree, 2000; Malone et al. 2000; Savary et al. 2010), which

necessitates the examination of the impacts of changing WA on flavor and consumer perception of beverages.

## **2.2. Beverage Flavor Emulsions Overview**

The essential flavor oils used in beverages contain water insoluble compounds which, due to immiscibility, present a challenge in water-based beverages. To adjust for immiscibility, an emulsion is utilized to disperse the flavor oil throughout the beverage (Tan, 2004). Beverage flavor emulsions not only add aroma, but also a cloudy appearance and color. A cloud emulsion is an alternative type of beverage emulsion which uses emulsified, flavorless vegetable oils to produce a desirable cloudy appearance (McClements, 2016). In these two-phase emulsions systems, the lipids are the dispersed phase, and the water is the continuous phase.

### **2.2.1 Composition and Usage Levels**

In a typical beverage formulation, flavor oil comprises a very small portion of the product. The main component of beverages is water; in a conventional soda, water accounts for 85-90% (w/w) and even more in diet sodas (Tan, 2004; Taylor, 2007). Common ranges of sugar, emulsifier, and other additives of beverage formulations are shown in Table 2.2. Other ingredients may include artificial sweeteners (for diet sodas), acidulants (citric acid and malic acid), preservatives (sodium benzoate), colorants, carbonation (carbon dioxide), or even alcohol (ethanol) (McClements, 2016). Flavor oils comprise approximately 0.015% (w/w) of the final beverage, although the flavor oil level for a citrus beverage can vary between 0.01 to 0.5% (100 - 5000 ppm) (Trubiano, 1995; Taylor, 2007).

Prior to the final beverage formulation, a flavor emulsion is typically created as a stock solution and diluted in the final beverage. This flavor emulsion contains a high amount

(approximately 10% w/w each) of oil phase (flavor and WA) and emulsifier, with common ranges shown in Table 2.2. In this concentrated form, the oil phase is dispersed and homogenized in an aqueous phase containing a dissolved emulsifier. This stock solution is typically produced in a central manufacturing facility, allowing the problematic procedures of emulsification and homogenization to be streamlined at one facility. Therefore, only simple mixing must subsequently take place at each of the bottling locations (Taylor, 2007). These centrally produced beverage emulsions contribute to more product consistency and simple processing at the bottling sites.

### 2.2.2 Approaches to Improve Emulsion Stability

Many factors contribute to emulsion stability, each inhibiting one or more of the mechanisms of phase separation. Within beverage emulsions, the most relevant equation to describe the key influences is Stokes' Law (Eq. 1) (McClements, 2016). The creaming velocity ( $v$ ) is estimated using the gravitational constant ( $g$ ), the droplet radius ( $r$ ), the difference in density between the dispersed and continuous phases ( $\Delta\rho$ ), and the continuous phase viscosity ( $\eta$ ).

$$v = \frac{-2 g r^2 (\Delta\rho)}{9 \eta} \quad [\text{Eq. 2.1}]$$

Based on this equation,  $\Delta\rho$  and  $r$  are the two controllable factors within a beverage emulsion. WAs are added to lessen the difference in densities, and homogenization is applied to reduce the droplet size of the emulsion. The gravitational constant ( $g$ ) is constant in any realistic beverage scenario, and the viscosity ( $\eta$ ) must remain low, approximately near that of water, to fit the expectations of a beverage.

The five commonly discussed modes of phase separation are creaming, sedimentation, flocculation, coalescence, and Ostwald Ripening (McClements, 2016; Tan, 2004), and these mechanisms of emulsion instability are graphically described in Figure 2.1. Creaming and sedimentation are both methods of gravitational separation, driven by a difference in density between the phases ( $\Delta\rho$ ) in accordance with Stokes law (Eq. 2.1). Creaming refers to the positional rise of dispersed phase droplets, while sedimentation refers to the descent of the dispersed phase droplets (McClements, 2016). Flocculation is a method of emulsion instability that consists of the aggregation of droplets, but not the incorporation of these droplets together (Tan, 2004). Coalescence and Ostwald Ripening are two methods of instability that occur through the combination of droplets, driven by an overall decrease in contact area between the phases (McClements, 2016). The increase in droplet size leads to emulsion instability through the droplet radius ( $r$ ) parameter of Stokes law (Eq 2.1). During coalescence, droplets combine material through the breakage of the interfacial droplet film to form one larger droplet. Alternatively, Ostwald Ripening is driven by the solubility of droplet material, promoting migration of material from small droplets to large, through the continuous phase (McClements, 2016). Within an emulsion, each of these mechanisms may impact the stability, and by knowing the driving factors, the instability can be addressed.

#### 2.2.2.1 Weighting Agents

A weighting agent (WA) is a high density compound dissolved in a flavor oil to increase the oil phase density and inhibit gravitational separation in beverages. Gravitational separation causes ringing (also known as creaming) which is the visible separation of a beverage to form an oil ring at the interface of water and air (Reynolds and Chappel, 1998). An ideal WA should be insoluble in water, have a higher density than water, and contribute

little to flavor or odor (Tan, 2004). Three WAs are shown in Figure 2.2 with relevant properties of each molecule described in Table 2.1. A WA is most commonly used with citrus oils (Table 2.3 and 2.4) due to the low density and immiscibility in water of these types of flavors. Orange oil has a density of 0.86 g/mL, WAs have a density between 1.08 and 1.33 g/mL, and a typical sugar solution used in a beverage has a density of 1.03 g/mL (for diet drinks the density is closer to water at 1.0g/mL) (Tan, 2004). Therefore, WAs are added to citrus oils to adjust for a total mixture density approaching 1.0 g/mL. The usage level to reach a sufficient density requires the WA to comprise 10-40% (w/w) of the oil phase. The densities of common WAs, along with common and regulatory usage levels, are listed in Table 2.1. Because the density of BVO is higher than either SAIB or EG, larger amounts of the SAIB or EG are required to match the same oil phase density as BVO.

The usage of WAs contribute an entropy of mixing effect within the oil phase and thus also stabilize emulsions by inhibiting the Ostwald Ripening (Lim et al. 2011). By decreasing the solubility of the oil, the material within a droplet is less susceptible to enter the continuous phase, and the growth of droplets by Ostwald Ripening is slowed. (Li et al. 1998; Chang et al. 2012). Therefore, WAs not only stabilize beverage emulsions through the intended mechanism of increasing oil phase density, but also by slowing Ostwald Ripening of the oil droplets. The approved weighting agents in the United States, SAIB, BVO, and EG, all have a density greater than water, but are chemically different.

#### 2.2.2.1.1 Sucrose Acetate Isobutyrate (SAIB)

SAIB is a WA permitted in the United States for usage in beverages below the level of 300 ppm (21 C.F.R. § 172.833, 2015; Eastman Chemical Company, 2010). Numerous studies have assessed the potential toxicity of this ingredient prior to FDA approval for safe

usage in 1999 (Reynolds, 1998; 21 C.F.R. § 172.833, 2015). SAIB can be produced by reacting sucrose with isobutyric and acetic anhydrides, followed by several steps of purification (Reynolds and Chappel, 1998). Due to the synthesis method, SAIB is not a pure compound but instead a mixture of esterified sucrose molecules with a distribution of ester moieties (Reynolds, 1998). The average distribution of esters on each sucrose is two acetate and six isobutyrate, and this molecule is shown in Figure 2.2A (Reynolds, 1998).

Interestingly, this sucrose ester was proposed as a liquid phase in a gas chromatographic method for separating flavor compounds (Smith et al. 1960). SAIB is also marketed as a fixative in cosmetics to extend the aroma and fragrance in products such as nail polish and perfumes (Eastman Chemical Company, 2013). These alternative usages suggest the presence of selective interactions between this molecule and some flavor compounds.

In beverages, SAIB is commonly used at concentrations of 50-300 ppm, depending on the density and amount of flavor required (Eastman Chemical Company 2010 ; Reynolds and Chappel 1998). Multiple studies have demonstrated the effectiveness of SAIB in stabilizing emulsions (Trubiano 1995; Chanamai and McClements, 2000). SAIB has a density of 1.146 g/cm<sup>3</sup> and is a highly viscous liquid (100,000 cP at 30°C) (Eastman Chemical Company, 2010). Due to the high viscosity, SAIB can be difficult to manipulate during manufacturing. Consequently, SAIB can also be purchased preblended with medium chain triglyceride (MCT) oil, ethanol, or citrus oil which all have lower solution viscosities (Eastman Chemical Company, 2010). As of 2017, SAIB is currently used in numerous national beverage products as evidenced by various ingredient statements (PepsiCo's "Gatorade® Orange," "Mountain Dew® Kickstart Orange Citrus," and CocaCola's "Powerade® Tropical Mango").

#### 2.2.2.1.2 Brominated Vegetable Oil (BVO)

BVO is a thick, high density oil which has been widely used as a WA in citrus beverages since 1958, when it was first accepted by the United States FDA (21 C.F.R. § 180.30, 1984). This ingredient can be produced by the addition of aqueous bromine to vegetable oil, agitation, and subsequent purification (Bendig et al. 2012). Vegetable oil is a natural mixture of many chain lengths with varying degrees of unsaturation, thus the composition of BVO is also a blend of brominated triglycerides. Predominantly, BVO is composed of glycerol attached to one linoleic acid with four brominated carbons ( $C_{18}H_{28}Br_4O_2$ ) and two oleic acids with two brominated carbons, as shown in Figure 2.2B (Bendig et al. 2012). The bromine atoms in this molecule bring significant polarizability, producing greater interactions between molecules, as evidenced by the higher viscosity than plain vegetable oil (Thomas et al. 2015). This polarizability suggests that there may be interactions present between flavor compounds and this WA within the oil phase of a beverage application.

In the European Union, BVO is not permitted in beverages, but the United States allows BVO usage although it cannot exceed 15 ppm in finished beverages (21 C.F.R. § 180.30, 1984). With a density of  $1.33 \text{ g/cm}^3$ , BVO is typically used at lower concentrations (8 ppm) than SAIB (50 ppm). Experimental studies have demonstrated the effectiveness of BVO in stabilizing emulsions (Chanamai and McClements, 2000). Despite recent concerns over the presence of brominated ingredients in beverages, BVO is utilized in many commercial products as of 2017, such as PepsiCo's iconic "Mountain Dew®."

#### 2.2.2.1.3 Glycerol Ester of Wood Rosin "Ester Gum" (EG)

EG is a resin whose usage has increased recently with the trend of removing BVO from beverage formulations. At room temperature, this product is solid and has a density of 1.08 g/cm<sup>3</sup>. Synthesis of the product involves esterification of glycerol with a wood rosin which, due to the variability of molecules within wood rosin, creates a mixture of compounds instead of a pure compound ingredient (Tan, 2004). EG is composed, on average, of a thrice esterified glycerol with abietic acid as displayed in Figure 2.2C (Tan, 2004).

In the United States, EG cannot exceed 150 ppm in a finished beverage and is labeled with the name 'glycerol ester of wood rosin' (21 C.F.R. § 172.735, 2007). Typical usage concentrations are 50-100 ppm, fluctuating based on the type and amount of flavor. Previous studies have demonstrated the ability of EG to stabilize emulsions (Tse and Reineccius, 1995; Chanamai and McClements, 2000; Lim et al. 2011). Unfavorably, EG is prone to oxidation which changes the aroma and solubility into an aqueous phase (Tan, 2004).

#### 2.2.2.2 Emulsifiers

By far, the most commonly used emulsifier in beverage emulsions is gum acacia (also known by the name arabic gum, GA). Emulsifiers are added to emulsions to decrease the surface tension between the oil and water phases and inhibit coalescence of the emulsion. Once a larger droplet has formed, the system is more prone to gravitational separation. Additionally, emulsifiers such as gum acacia enhance homogenization by acting as a mechanical barrier against coalescence and increasing density of the droplet by film formation (Trubiano, 1995).

Gum acacia is a 2500 kDa glycoprotein composed of approximately 10% proteinaceous material, and the remainder is polysaccharide based (Randall et al. 1988; Aokia et al. 2007). A high concentration of the protein in gum acacia is concentrated in one

of the polymer subunits (Randall et al. 1988). Due to the small proportion of protein (which was shown to provide the surface-active properties), only 1-2% of the gum acacia molecule is adsorbed at the emulsion interface (Randall et al. 1988). This low surface interaction ability requires a relatively high usage level (of approximately equal parts gum acacia to the oil phase), to form a stable emulsion (Aokia et al. 2007). Although gum acacia has historically been widely used as the emulsifier in beverage emulsions, research has been conducted recently to replace it with more consistent and cost effective products, such as hydrophobically modified starch, proteins, polysaccharides, and other surface active molecules (McClements, 2016).

#### 2.2.2.3 Homogenization

An additional method to stabilize beverage emulsions is homogenizing the mixture to decrease the droplet size, creating a smaller and more uniform size distribution. To form the initial emulsion, a primary homogenization must be performed, and a secondary homogenization step further decreases the particle size (McClements, 2016). The final droplet size is influenced by both the amount of surfactant and the total shear work, which can be calculated through summation of all work during each pass through the homogenizer (Steffe and Daubert, 2006). Homogenization is an important method in stabilizing an emulsion, but to produce the required shelf life of citrus oil beverages, WAs must be employed in tandem.

### **2.3. Citrus Flavor Oil Composition**

Most citrus flavoring utilized in commercial beverages is added in the form of an essential oil. A citrus essential oil is formed through the pressing or distillation of fruit peels and is often combined with further extraction to concentrate the flavor active compounds in a

process known as folding (ISO 9235, 2013; Owusu-Yaw et al. 1986). The composition of citrus essential oils (orange, lemon, and lime) consists of mostly the same compounds, in varying ratios, comprised of mostly terpenes and their decomposition or oxidation products (Shaw, 1979; Turek and Stintzing, 2011; Hognadottir and Rouseff, 2003; Hinterholzer and Schieberle, 1998; Vora et al. 1983; Perez-Cacho and Rouseff, 2008, Qiao et al. 2008). The characteristic differences between citrus flavors originate from numerous compounds at specific ratios (Shaw, 1979; Perez-Cacho and Rouseff, 2008). These essential oils can contain hundreds of compounds, but not every compound contributes to perceived flavor (Hinterholzer and Schieberle 1998; Buettner and Schieberle, 2001; Qiao et al. 2008; Xiao et al. 2016). For example, Hognadottir and Rouseff (2003) found that only 34% of identified molecules in essential orange oil had volatile activity which related to 25 of the 74 molecules detected by GC-MS. Additionally, components of essential oils are readily reacted by oxidization and polymerization which can lead to off flavors and change in color (Owusu-Yaw et al. 1986; Turek and Stintzing, 2013). Acidic environments, like those in carbonated beverages, have been demonstrated to convert limonene and linalool to other flavor molecules through simple rearrangements (Hausch et al. 2015). Among the many compounds identified in these oils, a truncated group of molecules is consistently identified, reported, and attributed to the characteristic flavor.

The main volatile constituents of essential citrus oils can be broken down into groups based on chemical classifications (Table 2.3 and 2.4). Although limonene is consistently reported as the major component of orange oil (by weight) it is reported to have minimal flavor activity (Tamura et al. 1996; Marin et al. 1992; Hognadottir and Rouseff, 2003). Instead, the oxygenated compounds (such as linalool, decanal, and octanal) are typically the

most flavor active constituents of the citrus essential oils (Rouseff and Perez-Cacho, 2007; Kostadinovic et al. 2010). The hydrocarbon, non-oxygenated molecules comprised of multiple isoprene units are denoted as the *terpene* group. These *terpenes*, which include limonene,  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene, and  $\gamma$ -terpinene, have high threshold concentrations, and as such, are typically considered to have minimal flavor activity (Perez-Cacho and Rouseff, 2008). Alternatively, the *terpenoid* group describes terpene molecules which have been oxygenated and contain an aldehyde group. Some of these *terpenoids* represent oxygenated off-flavors, while others are considered key compounds (like citral) (Choi et al. 2009). Compounds also containing an aldehyde group, but with a straight-chained carbon chain instead of isoprene units, are designated *saturated aldehydes*. In this group, hexanal and octanal are correlated with a ‘green’ aroma and decanal with a ‘peely’ aroma (Xaio et al. 2016). The *alcohol* group consists of molecules that have one hydroxyl group attached to the hydrocarbon structure. Linalool, an *alcohol*, is often cited as a key compound in citrus flavors (Marin et al. 1992; Perez-Cacho and Rouseff 2008) although  $\alpha$ -terpineol and terpinen-4-ol are considered off flavors from limonene oxidation (Shaw, 1979). These four classes of volatile flavor compounds include many of the relevant flavor molecules that make up each of the citrus flavors.

### 2.3.1 Orange Oil

Due to the wide popularity of orange flavor and its essential oil, this flavor blend has been widely characterized and studied. In orange oil, linalool is consistently reported as one of the most flavor active compounds due to the low threshold value and contributable concentration (Marin et al. 1992; Perez-Cacho and Rouseff, 2008) as well as octanal (Tamura et al. 1996) and decanal (Buettner and Schieberle, 2001). In orange juice, important flavor

compounds were hexanal, heptanal, octanal, decanal, citral, citronellal,  $\alpha$ -pinene,  $\beta$ -myrcene, limonene, linalool, and  $\alpha$ -terpineol (Hognadottir and Rouseff, 2003; Mastello et al. 2015; Hinterholzer and Schieberle, 1998; Plotto et al. 2008). Additionally, some molecules such as  $\beta$ -ionone and wine lactone displayed high aroma activity but were not detectable by GC-MS, likely due to low concentration levels (Hognadottir and Rouseff, 2003). Other compounds in orange juice with high flavor activity were ethyl butanoate, ethyl 2-methylbutanoate, and acetaldehyde, although these compounds are not typically detected in orange oils (Plotto et al. 2008). Previous experiments with orange oil have utilized 10-15 representative flavor compounds which consisted of  $\alpha$ -pinene, ethyl butyrate,  $\beta$ -pinene, 3-carene, myrcene, limonene,  $\gamma$ -terpinene, octanal, decanal, linalool, neral and geranial (Mirhosseini et al. 2008; Mirhosseini and Tan, 2009; Mirhosseini et al. 2010). These key compounds, also present in other citrus flavors, are shown and described in further detail in Table 2.3 and Table 2.4.

### 2.3.2 Lemon Oil

Lemon essential oil is composed of many of the same compounds as orange oil, and it is the different ratios which contribute to the unique and characteristic taste (Shaw 1979; Allegrone et al. 2006; Turek and Stintzing, 2011). The nuances within lemon flavor were suggested to be dependent on the neral and geranial content in the oil, which is typically reported to be between 2 and 13% w/w (Shaw 1979; Kostadinovic et al. 2010). The flavor compounds  $\beta$ -pinene and  $\gamma$ -terpinene are also typically higher in lemon oil than other citrus oils (Shaw 1979; Rouseff and Perez-Cacho, 2007). Based on their flavor dilution values from a solvent extraction, linalool, geranial, and neral were determined to be the strongest aroma compounds in essential lemon oil (Schieberle and Grosch, 1988). Other compounds with flavor activity in lemon oil were myrcene, limonene,  $\gamma$ -terpinene, octanal, nonanal,

citronellal, 2-nonenal, and decanal (Schieberle and Grosch, 1988). Several of these compounds denoted as characteristic aromas in lemon, were also considered important in orange oil.

### 2.3.3 Lime Oil

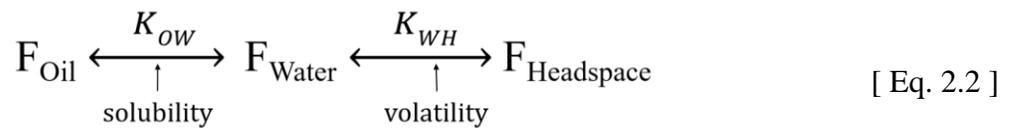
Involving many of the same molecules as orange and lemon oil, lime oil is also composed of mostly terpene molecules. Lime oil aroma is characterized by the key odorants geranial, neral, and linalool (Chisholm et al. 2003). The compounds comprising a majority of the monoterpene fraction, in order of weight percentage, are limonene,  $\beta$ -pinene,  $\gamma$ -terpinene, sabinene,  $\alpha$ -pinene, and myrcene (Mondello et al. 1995). Among the terpenes, only limonene,  $\alpha$ -pinene, and myrcene contributed to flavor while many others were present at levels below their thresholds (Chisholm et al. 2003). Linalool is present in three of the top lemon-lime carbonated beverages and had the highest flavor activity (Hausch et al. 2015). Other molecules in commercial lemon-lime beverages with high flavor activity, as determined through flavor dilution and odor-activity values, are octanal, nonanal, decanal, limonene, 1,8-cineole, neral, geranial,  $\alpha$ -terpineol, and geranyl acetate (Hausch et al. 2015). Based on the characteristic flavor compounds, lime oil appears to be more readily distinguished than the orange and lemon oils.

## 2.4. Molecular Interactions

### 2.4.1 Partitioning Coefficients

The partitioning of individual flavor molecules is one of the largest contributing factors governing the perception of flavor, as it describes the location of molecules in the headspace, liquid, or other phase of food (Overbosh et al. 1991; Taylor, 1998; de Roos, 2000; McClements, 2016). A typical flavor oil-in-water emulsion contains four main factors

predicted to affect flavor release: 1) oil content, 2) surface-active agent, 3) any additives in the product, and 4) structure or type of emulsion (Charles et al. 2000; McClements, 2016). The diagram of a model beverage emulsion, at a molecular size, is shown in Figure 2.3 to illustrate the key parameters involved in this system. The continuous phase of this emulsion is water (or sugar water), while the dispersed phase is a mixture of the flavor oil and the WA (Tan, 2004; McClements, 2016). The dispersed phase is stabilized by an emulsifier added to the liquid phase, but located at the interface of oil and water. According to the model, molecules within the aqueous phase are responsible for the ‘taste’ of a beverage, detected on the tongue as one of the five basic tastes (bitter, sweet, salty, sour, and umami) (Lawless and Heymann, 2010). Volatile molecules present in the air phase are responsible for the aroma or odor of the beverage, providing a characteristic flavor such as orange or grape (Lawless and Heymann, 2010). Relative equilibrium distributions of a compound between phases is described by a partition coefficient. Each flavor molecule has an equilibrium distribution between the hydrophobic and aqueous phases (solubility) and the aqueous phase and headspace (volatility). The relationship between these partition coefficients of oil components is shown in Eq. 2.2 and graphically in Figure 2.3.



The concentration of flavor compounds in the headspace is a factor of both equilibrium partition coefficients ( $K_{OW}$  and  $K_{WH}$ ) and the initial concentration of flavor in the oil droplet. The oil-water partition coefficient ( $K_{OW}$ ) represents the miscibility or solubility of a molecule

and is calculated by the ratio of the concentration of a compound in the oil divided by the concentration of that compound in the water (Eq. 2.3) (McClements, 2016).

$$K_{OW} = \frac{[F]_{oil}}{[F]_{water}} \quad [ \text{Eq. 2.3} ]$$

The water-headspace partition coefficient ( $K_{WH}$ ) represents the volatility of a molecules and is similarly calculated by the concentration of a compound in the water divided by the concentration of that compound in the headspace.

$$K_{WH} = \frac{[F]_{water}}{[F]_{headspace}} \quad [ \text{Eq. 2.4} ]$$

The partitioning between phases is dictated by the entropy of volatilization, the enthalpy of flavor-flavor bonds, the enthalpy of solvent-flavor interactions, and the temperature.

Therefore, the molecular properties of both the water and oil impact the partition coefficient.

Each molecule is expected to have unique partition coefficients based on the molecular interactions with each phase. The addition of new compounds to the oil phase modifies those molecular interactions and is thus likely to modify the partition coefficients due to binding with or repulsion of the flavor molecules (McClements, 2016). The partition coefficient does not consider the speed or kinetics at which equilibrium is achieved, but only the ratios of molecules once equilibrium has been established.

The kinetics of flavor release is another complex phenomenon which describes the speed at which the equilibrium partition coefficient is reached. Due to the dynamic environment during beverage consumption, the release of flavor in the mouth is influenced by mixing, dilution with saliva, mass transfer resistance, thermal instability, and eddy diffusion in addition to partition coefficients within a food (McNulty and Karel, 1973; Harrison et al. 1997; Voilley et al. 2000). These parameters must all be considered in an

accurate model for predicting flavor release during consumption of a beverage, leading to a high degree of complexity. This difficulty has led many researchers to consider only the equilibrium concentrations, using partition coefficients, instead of all the factors involved with the kinetics of flavor release (Harrison et al. 1997).

It should be noted that this three-phased equilibrium system does not follow ideal conditions, and thus the mathematical theories cannot be precisely applied. Henry's law is violated because of the multiple components jointly dissolved into the water; partition coefficients are non-ideal because the surface area of the emulsion droplets can bind flavors; and the numerous flavor compounds likely interact with each other (Taylor, 1998). However, these ideal rules provide the closest approximations of how the system interactions can be explained, although established mathematical models may be invalid (Taylor, 1998).

#### 2.4.2 Formation Constants ( $K_f$ )

The complex formation constant, or binding constant ( $K_f$ ), describes the relative extent of bound vs free molecules to a substrate and has been used to measure the association of flavor compounds to several ingredients (Overbosch et al. 1991; Harrison et al. 1997; McClements, 2016). The  $K_f$  can be calculated using the following equation adapted from Astray et al. (2010) and Rekharsky and Inoue (1998) where  $[F]_{\text{WA-Complex}}$  is the concentration of flavor in a WA complex,  $[F]_{\text{Free}}$  is the concentration of unbound flavor, and the  $[WA]$  is the WA concentration:

$$K_f = \frac{[F]_{\text{WA-Complex}}}{[F]_{\text{Free}} \times [WA]} \quad [ \text{Eq. 2.5} ]$$

Molecules with lower association to the WA produce smaller  $K_f$  values and highly interactive molecules produce larger  $K_f$  values. Calculation of the  $K_f$  can aid in the development of

predicting molecular interactions by providing a partition coefficient that considers the concentration of both the analyte (such as a flavor compound) and the ligand (such as a WA) (Landy et al. 2000). Therefore, this binding constant allows for comparison of a more universal affinity between molecules than previously utilized parameters, such as percent change or amount decreased from the control sample.

Cyclodextrin interactions with flavor compounds have been studied using  $K_f$  values to characterize their impacts as encapsulation agents in commercial flavor blends. By measuring changes in the volatility of flavor compounds with the inclusion of cyclodextrins (using  $K_f$ ), it was proposed that molecular properties could predict the extent of the interaction with the added compound (Reineccius et al. 2005). Additionally,  $K_f$  values of various flavor compounds were employed to find correlation with molecular properties to predict the driving factors of the molecular association (Astray et al. 2010). These compound properties which correlated with  $K_f$  were suggested to define the type of molecular interactions occurring between the ligand and analyte. The  $K_f$  value has been recognized as a measurement of flavor compound interactions and has even been used to model molecular associations.

#### 2.4.3 Statistical Modeling By Molecular Properties (QSAR)

Many parameters have been established to describe the molecular and physical properties of molecules, and these values can be useful in predicting molecular activity or function in a system. This process of predicting the response of a compound, based on molecular properties, describes quantitative structure activity relationships (QSAR) (Landy et al. 2000). Using molecular structures, models or rules can be developed to predict activity based purely on a few selected properties. Hundreds of properties can be calculated for a

single molecule, and many show correlation to the response, but often several of them have multicollinearity. Therefore, this high correlation to each other makes it redundant to include all the parameters in one model. For example, molecular weight, volume, diameter, and boiling point displayed high correlation to each other and, similarly, another group of high correlation was the Henry constant estimation, partition coefficient in water, partition coefficient in gum solution, and diffusion coefficient in water (Savary et al. 2014). Due to collinearity, only one parameter from each of the groups needed to be included in the final model for a good prediction of activity.

Some of the commonly used molecular properties are the octanol-water partition coefficient ( $\log P$ ), vapor pressure, molar volume ( $V_m$ ), and molar refractivity ( $A$ ) (van Ruth et al. 2000; Astray et al. 2010; Landy et al. 2000). The  $\log P$  is the logarithm of the partition coefficient between octanol and water ( $K_{OW}$ ), and it is calculated by quantifying the compound in each phase of a two-phase mixture (McClements, 2016). Instead of conducting tedious experiments, many  $\log P$  values are estimated based on the molecular structure of a compound (Tromelina and Guichard, 2004). Molar refractivity ( $A$ ) is a measure of the polarizability of the molecule and describes the volume of relative polarizability in one mole. Both  $\log P$  and  $A$  have been used in QSAR analysis, but the most widely utilized parameter to predict flavor compound interactions has been  $\log P$  (Charles et al. 2000; Carey et al. 2002; van Ruth et al. 2002; Reineccius et al. 2005; Mirhosseini and Tan 2009; Astray et al. 2010; Savary et al. 2014). The  $\log P$  parameter was used to predict the binding of various flavors to cyclodextrins, and due to its high correlation, the hydrophobic/hydrophilic interactions were suggested to be the driving factor in the interaction (Astray et al. 2010). The calculation of

molecular properties and application of QSAR provides a powerful tool for predicting and understanding molecular interactions.

#### 2.4.4 Statistical Clustering

Variables can be grouped into clusters of similarity and correlation through statistical clustering to simplify or summarize a complex data set. Clustering is a data compression technique for reducing the number of variables in a data set to a more comprehensible level. This type of analysis is often useful for describing large numbers of variables in more generalized terms and for emphasizing the similarities and largest variances within the system (Martínez-Mayorga et al. 2011; Spencer et al. 2016). For example, Spencer et al. (2016) used hierarchical clustering to group 99 flavor attributes into more general groups (such as fruity and roasted), which then allowed for more meaningful generalizations to describe samples. This statistical analysis can be utilized to group the flavor compounds by their changes with addition of each WA to elucidate general interactions between these ingredients.

### **2.5. Flavor Changes Observed with Sample Modifications**

Many factors affect the perception of flavor in a food, including physiochemical, physiological, and psychological aspects (McClements, 2016). Physiochemical parameters will be addressed in this thesis, including molecular interactions between flavors and matrix. The physiological factors involved in flavor perception dictate the processing of sensorial signals and include the nasal receptors and neural pathways to the brain (McClements, 2016). Psychological factors include influences of the expectations or complex perceptions of a person when consuming a product. Psychological factors can change the perception of flavor, when physiochemical and physiological factors remain unchanged. These three factors are

expected to impact the perception of flavor in a beverage, but the influences of physiological and psychological parameters remain outside the scope of this thesis.

### 2.5.1 Ingredient Modifications

Switching between ingredients in product formulation modifies molecular interactions with flavor compounds and is often responsible for unintended changes in product flavor (Hatchwell, 1996; Deibler and Acree, 2000; Malone et al. 2000). Through ingredient modifications, flavor changes have been documented with various substitutions in emulsions (Charles et al. 2000; Chang 2012) and beverages (Deibler and Acree, 2000; Savary, 2010). For emulsion systems, modifying the concentration or identity of several components has been demonstrated to impact flavor release (Donsi et al. 2012; Moghimi et al. 2016). These modifications represent alterations to the equilibrium flavor partitioning due to binding between flavor compounds and select ingredients (Overbosch et al. 1991). This section will summarize published experiments on the impacts of changes in commonly used ingredients used in flavor emulsions.

#### 2.5.1.1 Oil Phase Interactions

The presence of a hydrophobic additive, such as corn oil in solid foods or a weighting agent in beverage emulsions, can modify the partition coefficient of flavor compounds (McNulty and Karel, 1973). A hydrophobic additive has been shown to decrease the solubility of flavor compounds into an aqueous phase (Malone et al. 2000; Philippe et al. 2003; Leksrisompong et al. 2010; Arancibia et al 2011). Modification in flavor compound partitioning has been hypothesized to occur through an increase in the entropy of mixing in the oil phase (McClements et al. 2012). In application, modifications to the amount of oil changed flavor compound partition coefficients and threshold concentrations in a model

emulsion (Leksrisompong et al. 2010). These changes in partitioning with oil additive concentration have even been observed to modify the perceived citrus flavor and linalool concentration in model emulsions (Arancibia et al. 2011). Within an orange juice matrix, the addition of 0.1% oil decreased the perceived terpene-like odor and increased the fruity odor (Buettner and Schieberle, 2001). Changes in miscibility suggested that increasing the amount of hydrophobic materials, such as the addition of WAs to an emulsion, would decrease the solubility of hydrophobic flavor compounds.

Not only does the amount of lipid impact flavor interactions, but the type of oil or fat also plays a role in partition coefficients (Fabre et al. 2006; Philippe et al. 2003; Chang et al. 2012; Mao et al. 2014). An emulsion with a lipid phase of triacetin had a higher concentration of aqueous citral than the same amount (by weight) of MCT oil (Choi et al. 2009). Other studies have shown differences in oil component solubility between soybean oil and MCT oil (Mao et al. 2014) and corn oil and MCT oil (Chang et al. 2012). The physical state of the lipid has also been demonstrated to impact partition coefficients; utilizing the same type and amount of emulsion lipid phase (3% by weight octadecane), there was a higher concentration of citral in the aqueous phase with a lipid in a solid phase than a liquid phase (Mei et al. 2010). Overall, these modifications in release of flavor from emulsions suggest both the quantity and chemical nature of the dispersed lipid phases impact flavor solubility and volatility.

#### 2.5.1.2 Beverage Additive Interactions

In addition to the impacts of oil phase, other studies have focused on the effects of different types or concentrations of aqueous additives (sweeteners and emulsifiers) in beverage emulsions. In beverages, gum acacia has been demonstrated to affect several

attributes of emulsions, including droplet size, stability, and flavor release. The presence of gum acacia at a concentration of 5% or higher was shown to significantly decrease the volatility of very lipophilic compounds (with a  $\log P > 3.6$ ), while less hydrophobic molecules were not significantly different with gum acacia concentrations up to 20% w/w (Savary et al. 2014). Therefore, hydrophobic compounds can be expected to be retained by solutions containing gum acacia at concentrations above 5%. Considering beverage emulsions contain gum acacia up to 20%, this relationship would be expected to have significant impact on flavor perception. Additionally, Savary et al. (2010) showed that the concentration of gum acacia modified the volatility of  $\alpha$ -terpineol and ethyl decanoate. This interaction was suggested to result from hydrophobic interactions between the flavor compound and the gum acacia, as there was no significant difference in viscosity (Savary et al. 2010). Many studies have shown that the concentration of emulsifier modifies both the solubility of compounds as well as the droplet size of the emulsions, and these impacts will be discussed in section\_2.5.2 (Processing Modifications).

In addition to the impact of the emulsifier concentration in a beverage, the type of emulsifier has also impacted flavor solubility. When oil emulsions containing flavor compounds and oil were homogenized with different types of emulsifiers, the aqueous concentration of several flavor compounds were significantly different (Donsi et al. 2012). The water concentration of limonene (originating in the oil phase) was increased from 0.021 mg/g to between 0.2 and 0.4 mg/g depending on the emulsifier (Donsi et al. 2012). Additionally, switching between protein emulsifiers in salad dressings (Charles et al. 2000) and modified starch and whey protein isolate in flavor emulsions (Cheong et al. 2014), modified the headspace concentration of some flavor compounds. In other systems, a

significant change in volatility was not observed when the emulsifier was switched from modified starch to gum acacia (Carey et al. 2002). The mechanism by which an emulsifier modifies flavor has been proposed to be both an association with compounds, or an alteration of the homogenization process which subsequently modifies flavor solubility.

### 2.5.2 Processing and Droplet Size Modifications

In addition to the impacts of direct changes in the formulation of a beverage, processing of the emulsion has been observed to impact the release of flavor molecules. Specifically, the arrangement of an oil and water system plays a role in the solubility of components in the oil phase into the water phase. This macro-structure is determined by the homogenization of the beverage, a process that forms oil droplets and reduces the size. With a constant process, there is a critical limit of emulsifier below which the surfactant concentration controls the particle size distribution (Yurgec et al. 2010) and above which the shear history controls the particle size distribution (McClements, 2016). Therefore, at a sufficient concentration of emulsifier, a higher pressured homogenization step would produce a smaller particle-sized emulsion.

The size of the oil phase droplets within the aqueous environment can modify the partition coefficient of oil components. The concentration of oil components in the water phase is much greater in an emulsified mixture than a separated two-phased system (Donsi et al. 2012; Moghimi et al. 2016). Furthermore, the particle size of the emulsion also impacts the miscibility of oil compounds. Under the assumption of the same formulation, a smaller droplet-sized emulsion resulted in a larger partition coefficient (a larger miscibility) of hydrophobic flavor compounds (Chang et al. 2012; Moghimi et al. 2016). The partition coefficients of all compounds were not equally impacted, and it appeared that smaller

droplets increased hydrophobic compounds solubility while more hydrophilic compounds (logP between 0.25 and 2.37) showed decreased solubility (Charles et al. 2000; Van Ruth et al. 2002). Smaller oil droplets (particle size between 7 and 15  $\mu\text{m}$ ) in salad dressings modified the concentration of aromatic compounds, and more specifically, non-polar molecules were increased in the headspace while polar groups were decreased (Charles et al. 2000). Droplet size differences have been hypothesized to change the partition coefficients of flavor compounds through less interaction with the emulsifier (since more is located at larger surface area of smaller particles) or a larger LaPlace pressure (created through greater curvature of the droplet) (Charles et al. 2000; Qian and McClements, 2011). The complex interactions between oil, emulsifier, and homogenization process all have been shown to impact the emulsion droplet size, further complicating the implications of each on flavor partition coefficients.

## **2.6. Flavor Perception Measurements**

### **2.6.1 Overview of Aroma and Taste Perception**

The overall flavor of a beverage is perceived through a variety of sensors in the mouth and olfactory system. On the tongue, there are receptors for detecting the five basic tastes (bitter, sweet, salty, sour, and umami) (Lawless and Heymann, 2010). To be perceived as an aroma or odor, a molecule must be present in the headspace and detected by the olfactory bulb in the nasal cavity (Lawless and Heymann, 2010). Since only the five basic tastes are perceived by the tongue, most characteristic flavors in a beverage are controlled through volatile molecules detected by the olfactory system. For an unbiased, instrumental method of flavor characterization, the utilization of a gas chromatograph - mass spectrometer (GC-MS) has often been used for identification and quantification of specific molecules in

food products. Additionally, sensorial descriptions by both untrained and trained panelists have been used to characterize and compare flavors in food products. When used in tandem, these techniques allow for identification, quantification, and determination of the impact of flavor and ingredient changes.

### 2.6.2 Headspace Gas Chromatography with Mass Spectrometry

Since volatile molecules are responsible for much of the flavor in a food product, many researchers utilize headspace (HS) gas chromatography (GC) with a mass spectrometry detector (MS) to analyze food products. The HS method consists of the injection of a sample in the gaseous phase, typically with a syringe, onto a GC column. Gas chromatography is a type of partition chromatography in which compounds are separated as they pass through a polymeric stationary phase and gaseous (often helium) mobile phase over the length of a column. Partitioning between these two phases, in addition to the gradual increase of temperature, separates the molecules contained in the sample by boiling point and hydrophobicity. As compounds exit the column, the MS detector ionizes, fragments, and counts fragments of molecules. Each molecule has a unique fragment pattern which can be used to identify compounds, and the number of fragments is linearly related to the concentration in the samples, which can be used for quantification. In application, HS GC-MS has been used in detecting and quantifying citrus oil components (Mirhosseini et al. 2007; Qiao et al. 2008; Xaio et al. 2015). Several researches have also utilized this technique to measure flavor changes in beverage emulsions through various ingredient modifications (Deibler and Acree 2000; van Ruth et al 2002). Additionally, HS-GC has been used to measure changes in volatility of flavor compounds with the inclusion of cyclodextrins (Reineccius et al. 2005; Ciobanu et al. 2013). For measurement of the complexation of highly

volatile compounds with minimal water solubility, HS-GC was suggested to be more sensitive to differences than solubility measurements (Ciobanu et al. 2013). For analysis of volatile compounds in food, HS GC-MS is one of the most preferred and frequently used methods.

### 2.6.3 Trained Panel Sensory Analysis

A commonly used tool in the sensory field is the quantitative analysis of flavor attributes by a panel of highly trained human tasters. Also termed descriptive analysis, this type of analysis allows for the identification and quantification of any number of attributes that are determined to be relevant for a product (Lawless and Heymann, 2010). Typically, specific and defined attributes are chosen, extensively trained, and measured by each of the 6 to 12 panelists (Drake, 2007). The Spectrum Method of descriptive analysis has been previously used in the analysis of citrus beverages to elucidate the type and extent of differences between commercially available drinks (Leksrisompong et al. 2012). The utilization of trained panel analysis allows a more sensitive and analytical technique than consumer testing, but still allows for quantitative and reliable attribute information comparable to an analytical chemistry technique (Lawless and Heymann, 2010).

### 2.6.4 Consumer Sensory Difference Testing

In contrast to the trained panel analysis of beverages, consumer difference testing is typically less sensitive to a difference between samples but is more representative of the implication of a noticeable difference. This type of testing assesses if there is, or is not, a noticeable difference between samples (Drake, 2007). Panelists are not trained, but are selected from a group of typical consumers of that product. One of the most frequently used sensory difference tests, when there is not an anticipated attribute of difference, is the triangle

test (Drake, 2007). In a triangle test, a panelist is presented with three coded samples (two are the same and one is different) and asked to choose which one is different from the other two (Lawless and Heymann, 2010). The samples are considered different when the number of correctly chosen samples is significantly greater than 33% (pure chance with three samples) (Lawless and Heymann, 2010). This test is ideal for assessing whether consumers of a product are likely to notice a difference when an ingredient is modified or replaced.

## **2.7. Conclusion**

For many years, WAs have been used in beverages without a full understanding of their impacts on the flavor system. Most research regarding WAs has focused on the effects of these ingredients on emulsion stability (Chanamai and McClements, 2000; Lim et al. 2011). The impacts of other beverage ingredients on flavor volatility, such as the emulsifier and oil content has been examined (Mirhosseini and Tan, 2009; Mirhosseini et al. 2008), but no known research has been published on the effects of the WAs SAIB, BVO, or EG on flavor compound volatility or solubility. Various model systems have been proposed as methods for analyzing types of interactions between flavor compounds and food additives. Essential citrus oil research has suggested a list of key flavor compounds which contribute to characteristic citrus flavors. Identification of flavor compounds that interact with WAs, along with a measure of the extent of these interactions, would assist beverage manufacturers to reformulate WAs without changing the consumer perception of the product.

## 2.8. References

- Allegro G, Belliardo F, Cabella P. 2006. Comparison of volatile concentrations in hand-squeezed juices of four different lemon varieties. *J.Agric.Food Chem.* 54(5):1844-8.
- Aoki H, Katayama T, Ogasawara T, Sasaki Y, Al-Assaf S, Phillips GO. 2007. Characterization and properties of *Acacia senegal* (L.) Willd. var. Senegal with enhanced properties (*Acacia* (sen) SUPER GUM™): Part 5. Factors affecting the emulsification of *Acacia senegal* and *Acacia* (sen) SUPER GUM™. *Food Hydrocoll.* 21(3):353-358.
- Arancibia C, Jublot L, Costell E, Bayarri S. 2011. Flavor release and sensory characteristics of o/w emulsions. Influence of composition, microstructure and rheological behavior. *Food Res.Int.* 44(6):1632-41.
- Astray G, Mejuto JC, Morales J, Rial-Otero R, Simal-Gándara J. 2010. Factors controlling flavors binding constants to cyclodextrins and their applications in foods. *Food Res.Int.* 43(4):1212-1218.
- Bendig P, Maier L, Vetter W. 2012. Brominated vegetable oil in soft drinks - an underrated source of human organobromine intake. *Food Chem.* 133(3):678-682.
- Buettner A, Schieberle P. 2001. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J.Agric.Food Chem.* 49(5):2387-94.
- Carey ME, Asquith T, Linforth RS, Taylor AJ. 2002. Modeling the partition of volatile aroma compounds from a cloud emulsion. *J.Agric.Food Chem.* 50(7):1985-90.
- Chanamai R, McClements DJ. 2000. Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J.Agric.Food Chem.* 48(11):5561-5.
- Chang Y, McLandsborough L, McClements DJ. 2012. Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening inhibitors. *J.Agric.Food Chem.* 60(48):12056-63.
- Charles M, Rosselin V, Beck L, Sauvageot F, Guichard E. 2000. Flavor release from salad dressings: sensory and physicochemical approaches in relation with the structure. *J.Agric.Food Chem.* 48(5):1810-6.
- Charles M, Lambert S, Brondeur P, Courthaudon J, Guichard E. 2000. Interactions of flavor compounds with food components - 28 Influence of Formulation and Structure of an Oil-in-Water Emulsion on Flavor Release. ACS symposium series. 763342.

Cheong KW, Tan CP, Mirhosseini H, Joanne-Kam WY, Sheikh Abdul Hamid N, Osman A, Basri M. 2014. The effect of prime emulsion components as a function of equilibrium headspace concentration of soursop flavor compounds. *Chemistry Central Journal* 8(1):1-11.

Chisholm MG, Wilson MA, Gaskey GM. 2003. Characterization of aroma volatiles in key lime essential oils (*Citrus aurantifolia* Swingle). *Flavour Fragrance J.* 18(2):106-15.

Choi SJ, Decker EA, Henson L, Popplewell LM, McClements DJ. 2009. Stability of citral in oil-in-water emulsions prepared with medium-chain triacylglycerols and triacetin. *J.Agric.Food Chem.* 57(23):11349-53.

Ciobanu A, Landy D, Fourmentin S. 2013. Complexation efficiency of cyclodextrins for volatile flavor compounds. *Food Res.Int.* 53(1):110-114.

de Roos KB. 2000. Physiochemical Models of Flavor Release from Foods. In: Anonymous Flavor Release. American Chemical Society. p 126-41.

Deibler KD, Acree TE. 2000. Effect of Beverage Base Conditions on Flavor Release. *ACS Symp.Ser.* 763333-341.

Donsì F, Annunziata M, Vincensi M, Ferrari G. 2012. Design of nanoemulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. *J.Biotechnol.* 159(4):342-350.

Drake MA. 2007. Invited Review: Sensory Analysis of Dairy Foods. *J.Dairy Sci.* 90(11):4925-4937.

Fabre M, Relkin P, Guichard E. 2006. Flavor Release from Food Emulsions Containing Different Fats. *ACS Symp.Ser.* (920):61-72.

Guichard E. 2015. 13 - Interaction of aroma compounds with food matrices. In: J. K. Parker, J. S. Elmore, L. Methven, editors. *Flavour Development, Analysis and Perception in Food and Beverages*. Woodhead Publishing. p 273-95.

Harrison M, Hills BP, Bakker J, Clothier T. 1997. Mathematical Models of Flavor Release from Liquid Emulsions. *Journal of Food Science -Chicago-* 62(4):653-658.

Hatchwell LC. 1996. Implications of Fat on Flavor. *ACS symposium series.* 63314.

Hausch BJ, Lorjaroenphon Y, Cadwallader KR. 2015. Flavor chemistry of lemon-lime carbonated beverages. *J.Agric.Food Chem.* 63(1):112-9.

Hinterholzer A, Schieberle P. 1998. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragrance J.* 13(1):49-55.

Högnadóttir A, Rouseff RL. 2003. Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *Journal of chromatography.A* 998(1-2):201-11.

Kostadinovic S., Stefova M., Nikolova D., Nedelcheva D., Martinez N., Lorenzo D., Dellacassa E., Mirhosseini H. 2010. Multivariate analysis discrimination of various cold-pressed lemon oils from different geographical regions. *Journal of Food, Agriculture and Environment* 8(2):117-121.

Landy D, Fourmentin S, Salome M, Surpateanu G. 2000. Analytical Improvement in Measuring Formation Constants of Inclusion Complexes between  $\beta$ -Cyclodextrin and Phenolic Compounds. *Journal of inclusion phenomena and macrocyclic chemistry* 38(1-4):187-198.

Lawless HT, Heymann H. 2010. *Sensory evaluation of food : principles and practices*. 2nd ed. ed. New York : Springer,. 1 online resource (xxiii, 596 pages) : illustrations. p.

Leksrisonpong PP, Barbano DM, Foegeding AE, Gerard P, Drake MA. 2010. The Roles Of Fat And Ph On The Detection Thresholds And Partition Coefficients Of Three Compounds: Diacetyl,  $\Delta$ -Decalactone And Furanol. *J.Sens.Stud.* 25(3):347-370.

Leksrisonpong PP, Lopetcharat K, Guthrie B, Drake MA. 2012. Descriptive Analysis Of Carbonated Regular And Diet Lemon-Lime Beverages. *J.Sens.Stud.* 27(4):247-263.

Li J, Perdue EM, Pavlostathis SG, Araujo R. 1998. Physicochemical properties of selected monoterpenes. *Environ.Int.* 24(3):353-358.

Lim SS, Baik MY, Decker EA, Henson L, Michael Popplewell L, McClements DJ, Choi SJ. 2011. Stabilization of orange oil-in-water emulsions: A new role for ester gum as an Ostwald ripening inhibitor. *Food Chem.* 128(4):1023-1028.

Malone ME, Appelqvist IAM, Goff TC, Homan JE, Wilkins JPG. 2000. A Novel Approach to the Selective Control of Lipophilic Flavor Release in Low Fat Foods. *ACS Symp.Ser.* 763212-229.

Mao L, Roos YH, Miao S. 2014. Flavour Release from Monoglyceride Structured Oil-in-Water Emulsions through Static Headspace Analysis. *Food Biophysics* 9(4):359-367.

- Marin AB, Acree TE, Hotchkiss JH, Nagy S. 1992. Gas chromatography-olfactometry of orange juice to assess the effects of plastic polymers on aroma character. *J.Agric.Food Chem.* 40(4):650-654.
- Martínez-Mayorga K, Peppard TL, Yongye AB, Santos R, Giulianotti M, Medina-Franco JL. 2011. Characterization of a comprehensive flavor database. *J.Chemometrics* 25(10):550-560.
- Mastello RB, Capobiango M, Chin S, Monteiro M, Marriott PJ. 2015. Identification of odour-active compounds of pasteurised orange juice using multidimensional gas chromatography techniques. *Food Res.Int.* 75281-288.
- McClements DJ, Henson L, Popplewell LM, Decker EA, Jun Choi S. 2012. Inhibition of Ostwald Ripening in Model Beverage Emulsions by Addition of Poorly Water Soluble Triglyceride Oils. *J.Food Sci.* 77(1):C33-C38.
- McClements DJ. 2016. *Food Emulsions : principles, practice, and techniques.* 3rd ed. Boca Raton : CRC Press, Taylor & Francis Group.
- McNulty PB, Karel M. 1973. Factors affecting flavour release and uptake in O/W emulsions: Release and uptake models. *J. Fd. Technol.* 8(1)309-318.
- Mei L, Choi SJ, Alamed J, Henson L, Popplewell M, McClements DJ, Decker EA. 2010. Citral stability in oil-in-water emulsions with solid or liquid octadecane. *J.Agric.Food Chem.* 58(1):533-6.
- Mirhosseini H, Salmah Y, Nazimah SAH, Tan CP. 2007. Solid-phase microextraction for headspace analysis of key volatile compounds in orange beverage emulsion. *Food Chem.* 105(4):1659-70.
- Mirhosseini H, Tan CP, Hamid NSA, Yusof S. 2008. Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion. *Food Chem.* 107(3):1161-72.
- Mirhosseini H, Tan CP. 2009. Response surface methodology and multivariate analysis of equilibrium headspace concentration of orange beverage emulsion as function of emulsion composition and structure. *Food Chem.* 115(1):324-333.
- Mirhosseini H, Tan CP, Kostadinovic S, Naghshineh M. 2010. Principle component analysis of equilibrium headspace concentration of beverage emulsion as function of main emulsion components. *J Food, Agriculture & Environment* 8(2):126-133.
- Moghimi R, Aliahmadi A, McClements DJ, Rafati H. 2016. Investigations of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food borne pathogens. *LWT - Food Science and Technology* 7169-76.

- Mondello L, Dugo P, Bartle KD, Dugo G, Cotroneo A. 1995. Automated HPLC-HRGC: A powerful method for essential oils analysis. Part V.† identification of terpene hydrocarbons of bergamot, lemon, mandarin, sweet orange, bitter orange, grapefruit, clementine and mexican lime oils by coupled HPLC-HRGC-MS(ITD). *Flavour Fragrance J.* 10(1):33-42.
- Overbosch P, Afterof WGM, Haring PGM. 1991. Flavor release in the mouth. *Food Rev.Int.* 7(2):137-184.
- Owusu-Yaw J, Matthews RF, West PF. 1986. Alcohol Deterpenation of Orange Oil. *J.Food Sci.* 51(5):1180-1182.
- Perez-Cacho PR, Rouseff RL. 2008. Fresh squeezed orange juice odor: a review. *Crit.Rev.Food Sci.Nutr.* 48(7):681-95.
- Philippe E, Seuvre AM, Colas B, Langendorff V, Schippa C, Voilley A. 2003. Behavior of flavor compounds in model food systems: a thermodynamic study. *J.Agric.Food Chem.* 51(5):1393-8.
- Plotto A, Margaría CA, Goodner KL, Baldwin EA. 2008. Odour and flavour thresholds for key aroma components in an orange juice matrix: esters and miscellaneous compounds. *Flavour Fragrance J.* 23(6):398-406.
- Qian C, McClements DJ. 2011. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocoll.* 25(5):1000-1008.
- Qiao Y, Xie BJ, Zhang Y, Fan G, Yao XL, Pan SY. 2008. Characterization of aroma active compounds in fruit juice and peel oil of Jincheng sweet orange fruit (*Citrus sinensis* (L.) Osbeck) by GC-MS and GC-O. *Molecules* (Basel, Switzerland) 13(6):1333-44.
- Randall RC, Phillips GO, Williams PA. 1988. The role of the proteinaceous component on the emulsifying properties of gum arabic. *Food Hydrocolloids* 2(2):131-40.
- Reineccius TA, Reineccius GA, Peppard TL. 2005. The effect of solvent interactions on alpha-, beta-, and gamma-cyclodextrin/flavor molecular inclusion complexes. *J.Agric.Food Chem.* 53(2):388-92.
- Reineccius G. 2006. *Flavor chemistry and technology.* Boca Raton: Taylor & Francis.
- Rekharsky MV, Inoue Y. 1998. Complexation Thermodynamics of Cyclodextrins. *Chem.Rev.* 98(5):1875-1918.
- Reynolds RC. 1998. Metabolism and pharmacokinetics of sucrose acetate isobutyrate (SAIB) and sucrose octaisobutyrate (SOIB) in rats, dogs, monkeys or humans: a review. *Food and*

chemical toxicology : an international journal published for the British Industrial Biological Research Association 36(2):95-9.

Reynolds RC, Chappel CI. 1998. Sucrose acetate isobutyrate (SAIB): historical aspects of its use in beverages and a review of toxicity studies prior to 1988. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 36(2):81-93.

Rouseff R, Perez-Cacho PR. 2007. Citrus Flavour. In: *Anonymous Flavours and Fragrances : Chemistry, Bioprocessing and Sustainability*. Berlin, Heidelberg : Springer Berlin Heidelberg. p 117-134.

Savary G., Hucher N., Bernadi E., Grisel M., Malhiac C. 2010. Relationship between the emulsifying properties of Acacia gums and the retention and diffusion of aroma compounds. *Food Hydrocoll.* 24(2-3):178-183.

Savary G, Hucher N, Petibon O, Grisel M. 2014. Study of interactions between aroma compounds and acacia gum using headspace measurements. *Food Hydrocoll.* 37:1-6.

Schieberle P, Grosch W. 1988. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J.Agric.Food Chem.* 36(4):797-800.

Shaw PE. 1979. Review of quantitative analyses of citrus essential oils. *J.Agric.Food Chem.* 27(2):246-257.

Spencer M, Sage E, Velez M, Guinard JX. 2016. Using Single Free Sorting and Multivariate Exploratory Methods to Design a New Coffee Taster's Flavor Wheel. *J.Food Sci.* 81(12):S2997-S3005.

Steffe JF and Daubert CR. 2006. *Bioprocessing Pipelines: Rheology and Analysis*. Freeman Press. East Lansing, MI, USA.

Tamura H, Fukuda Y, Padrayuttawat A. 1996. Characterization of Citrus Aroma Quality by Odor Threshold Values. *ACS Symp.Ser.* 637:282-294.

Tan, C. 2004. Beverage Emulsions. In: Larsson, K., & Friberg, S. *Food emulsions*. 4th ed., rev. and expanded. New York: M. Dekker. 485-524.

Taylor, B. 2007. Ingredients and Formulation of Carbonated Soft Drinks. In *Carbonated soft drinks: formulation and manufacture* (p.p. 48-86). Blackwell Publishing Ltd : Oxford, UK.

Taylor AJ, Pearson K, Hollowood TA, Linforth RST. 2009. Aroma Release at the Nano- and Microscale: Molecules to Droplets. *ACS Symp.Ser.* (1007):246-258.

- Taylor AJ. 1998. Physical chemistry of flavour. *Int.J.Food Sci.Tech.* 33(1):53-62.
- Thomas MJ, Bramblett KA, Green BD, West KN. 2015. Thermophysical and absorption properties of brominated vegetable oil. *Journal of Molecular Liquids* 211647-655.
- Tromelin A, Guichard E. 2004. 2D- and 3D-QSAR Models of Interaction between Flavor Compounds and beta-Lactoglobulin Using Catalyst and Cerius<sup>2</sup>. *QSAR & Combinatorial Science* 23(4):214-233.
- Trubiano PC. 1995. The Role of Specialty Food Starches in Flavor Emulsions. *ACS Symp.Ser.* 610199-209.
- Tse KY, Reineccius GA. 1995. Methods To Predict the Physical Stability of Flavor-Cloud Emulsion. *ACS Symp.Ser.* 610172-182.
- Turek C, Stintzing FC. 2013. Stability of Essential Oils: A Review. *Comprehensive Reviews in Food Science and Food Safety* 12(1):40-53.
- Turek C, Stintzing FC. 2011. Application of high-performance liquid chromatography diode array detection and mass spectrometry to the analysis of characteristic compounds in various essential oils. *Analytical and Bioanalytical Chemistry* 400(9):3109-3123.
- van Gemert LJ. 2011. Odour thresholds : Compilations of odour threshold values in air, water and other media (second enlarged and revised edition.). Utrecht, The Netherlands: Oliemans Punter & Partners BV.
- van Ruth SM, King C, Giannouli P. 2002. Influence of lipid fraction, emulsifier fraction, and mean particle diameter of oil-in-water emulsions on the release of 20 aroma compounds. *J.Agric.Food Chem.* 50(8):2365-71.
- van Ruth SM, O'Connor CH, Delahunty CM. 2000. Relationships between temporal release of aroma compounds in a model mouth system and their physico-chemical characteristics. *Food Chem.* 71(3):393-399.
- Voilley A, Diaz MAE, Druaux C, Landy P. 2000. Flavor Release from Emulsions and Complex Media. *ACS Symp.Ser.* 763142-152.
- Vora JD, Matthews RF, Crandall PG, Cook R. 1983. Preparation and Chemical Composition of Orange Oil Concentrates. *J.Food Sci.* 48(4):1197-1199.
- Xiao Z, Ma S, Niu Y, Chen F, Yu D. 2016. Characterization of odour-active compounds of sweet orange essential oils of different regions by gas chromatography-mass spectrometry, gas chromatography-olfactometry and their correlation with sensory attributes. *Flavour Fragrance J.* 31(1):41-50.

Yurgec MJ, Osborne J, Steffe JF, Daubert CR. 2012. Quantifying Shear Effects On A Model Emulsion System. *J.Food Process Eng.* 35(6):905-914.

Eastman Chemical Company, Eastman Sustain SAIB, Food Grade : Frequently Asked Questions, Publication ZM-100E, 2010.

Eastman Chemical Company, Eastman Sustain SAIB, for cosmetics and personal care, Publication CB-37G, 2013.

Particle Sciences, Emulsion Stability and Testing, Technical Brief :2011. Volume 2, 2011

Code of Federal Regulations. Title 21, sec. 172.833. (accessed Dec 1, 2015).

Code of Federal Regulations. Title 21, sec. 180.30. (accessed Dec 1, 2015).

Code of Federal Regulations. Title 21, sec. 172.735. (accessed Dec 1, 2015).

ISO. 2013. ISO 9235:2013 Aromatic natural raw materials -- Vocabulary. Geneva, Switzerland: ISO.

Table 2.1. Common weighting agents and information relevant to their usage in commercial beverages.

<b>Abbreviation</b>	<b>Nutrition Label Name</b>	<b>Density (g/mL)</b>	<b>Usage Levels</b>		
			<b>Typical</b>	<b>Maximum (FDA)</b>	<b>FDA Document</b>
SAIB	Sucrose Acetate Isobutyrate	1.14	50 ppm	300 ppm	21 CFR 172.833
EG	Glycerol Ester of Wood Rosin	1.08	50 ppm	100 ppm	21 CFR 172.735
BVO	Brominated Vegetable Oil	1.33	8 ppm	15 ppm	21 CFR 180.30

Source: Reineccius, 2006; FDA, CFR Title 21

Table 2.2. Typical soda formulation with a concentrated flavor emulsion (by weight percentages).

<b>Ingredient</b>	<b>Final Beverage</b>	<b>Flavor Emulsion</b>	<b>Functionality</b>
Water	85-90%	70-80%	Bulk Material
Sugar	10-15%		Sweetness (Sensory)
Gum Acacia	0.015%	10-20%	Stability of Flavor Emulsion
Citric Acid	0.03-0.05%		Acidity (Sensory and Preservative)
Flavor Oils	0.015%	5-10%	Flavor (Sensory)
Weighting Agent	8-100 ppm	0-5%	Stability of Flavor Emulsion
Sodium Benzoate	150 ppm		Preservative (Antimicrobial)
Potassium Sorbate	250 ppm		Preservative (Antimicrobial)

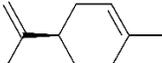
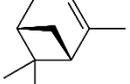
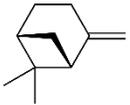
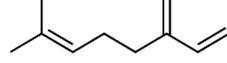
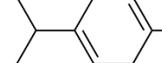
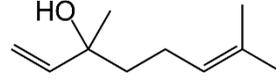
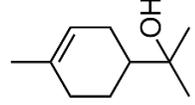
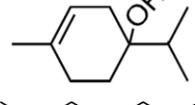
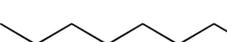
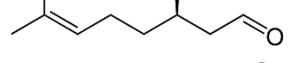
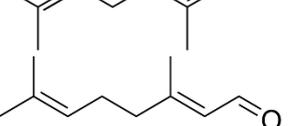
Source: Tan 2004; Steen and Ashurst 2006; Reineccius 2006

Table 2.3. Flavor Activity of Volatile Compounds Present in Orange Essential Oils

Compound	Class <sup>a</sup>	Odor		Odor Description
		Threshold [ppm]	Abundance (mg/mL) <sup>d</sup>	
Limonene	Terpene	0.01 <sup>b</sup>	650-720	Minty, lemon, citrus-like <sup>a</sup>
$\alpha$ -Pinene	Terpene	0.003 <sup>b</sup>	12-16	Resin, pine tree <sup>a</sup>
$\beta$ -Pinene	Terpene	0.14 <sup>b</sup>	0.1-0.4	Wood, terpene-like, pungent <sup>a</sup>
$\beta$ -Myrcene	Terpene	0.014 <sup>c</sup>	49 – 31	Mossy, musty <sup>a</sup>
$\gamma$ -Terpinene	Terpene	0.26 <sup>b</sup>	0.1-0.9	Sweet, citrusy <sup>e</sup>
Linalool	Alcohol	0.0001 <sup>b</sup>	16-2	Floral, sweet, fruity <sup>a</sup>
$\alpha$ -Terpineol	Alcohol	0.33 <sup>b</sup>	ND	Lemon, piney, minty <sup>e</sup>
Terpinen-4-ol	Alcohol	0.59 <sup>c</sup>	ND	Metallic <sup>a</sup>
Hexanal	Aldehyde	0.005 <sup>b</sup>	0.05-0.1	Green, grassy, soapy, fruity <sup>a</sup>
Octanal	Aldehyde	0.0007 <sup>b</sup>	2-8	Green, citrus-like, floral <sup>a</sup>
Nonanal	Aldehyde	0.001 <sup>b</sup>	0.5-1.6	Citrus-like, soapy, floral <sup>a</sup>
Decanal	Aldehyde	0.001 <sup>b</sup>	4-10	Green, citrus-like, fatty <sup>a</sup>
Citronellal	Terpenoid	0.006 <sup>b</sup>	0.5-2	Minty <sup>e</sup>
Citral (Neral)	Terpenoid	0.03 <sup>b</sup>	0.6-1.7	Lemon-like, minty <sup>a</sup>
Citral (Geranial)	Terpenoid	0.03 <sup>b</sup>	ND	Citrus-like, green, minty <sup>a</sup>

Sources: <sup>a</sup>Perez-Cacho and Rouseff, 2008; <sup>b</sup> van Gemert, 2011; <sup>c</sup> Tamura et al. 1996; <sup>d</sup> Xiao et al. 2016; <sup>e</sup>Hinterholzer and Schieberle, 2008. ND; not determined

Table 2.4. Chemical properties of key flavor compounds in citrus essential oils including the octanal:water partition coefficient (logP) and Molar Refractivity (A) in (cm<sup>3</sup>).

Compound	Classification <sup>a</sup>	Chemical Structure	logP <sup>b</sup>	A <sup>b</sup>
Limonene	Terpene		4.45	45.4
$\alpha$ -Pinene	Terpene		4.37	44
$\beta$ -Pinene	Terpene		4.37	43.8
$\beta$ -Myrcene	Terpene		4.58	47.6
$\gamma$ -Terpinene	Terpene		4.36	45.4
Linalool	Alcohol		3.28	49.5
$\alpha$ -Terpineol	Alcohol		2.79	47.1
Terpinen-4-ol	Alcohol		2.99	47.3
Hexanal	Aldehyde		1.97	30
Octanal	Aldehyde		3.03	39.3
Nonanal	Aldehyde		3.56	43.9
Decanal	Aldehyde		4.09	48.6
Citronellal	Terpenoid		3.48	48.4
Citral (Neral)	Terpenoid		3.17	48.4
Citral (Geranial)	Terpenoid		3.17	48.4

Sources: <sup>a</sup>Perez-Cacho and Rouseff, 2008; <sup>b</sup>ACD/Labs<sup>TM</sup> Estimate

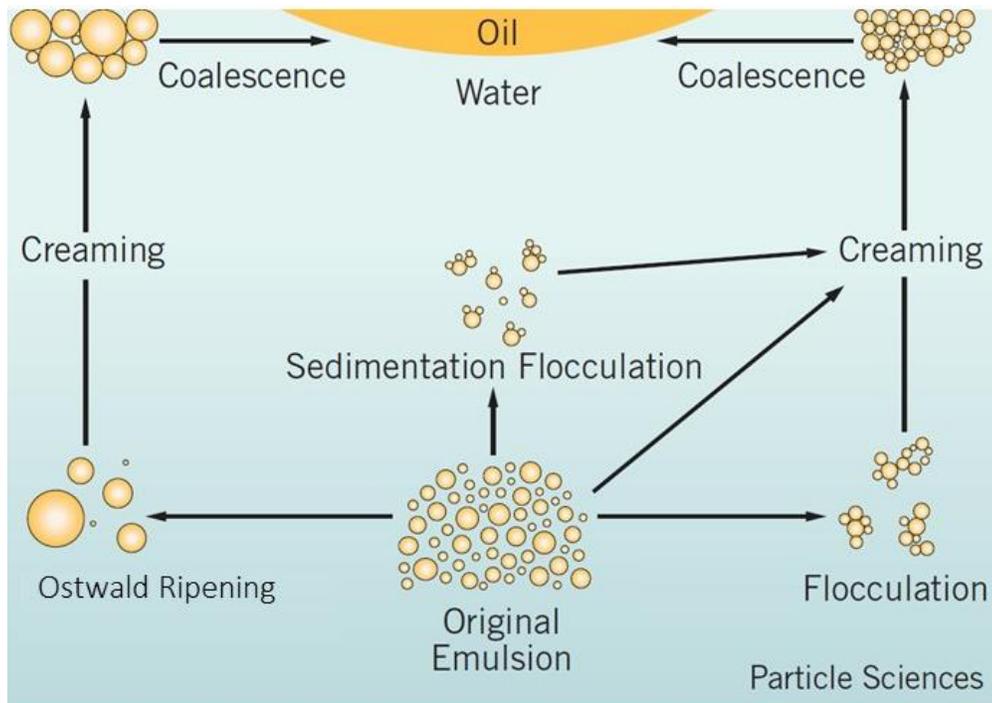
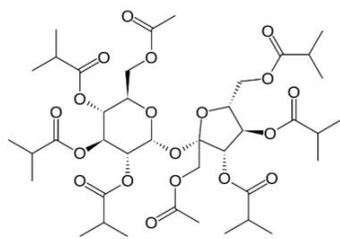
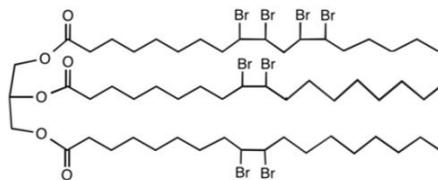


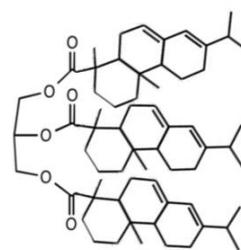
Figure 2.1. Mechanisms of physical instability in emulsions (adapted from Particle Sciences, 2011)



**A) SAIB**



**B) BVO**



**C) EG**

Figure 2.2. Chemical structures of the three most common weighting agents; A) sucrose acetate isobutyrate (SAIB) B) brominated vegetable oil (BVO) and C) ester gum (EG).

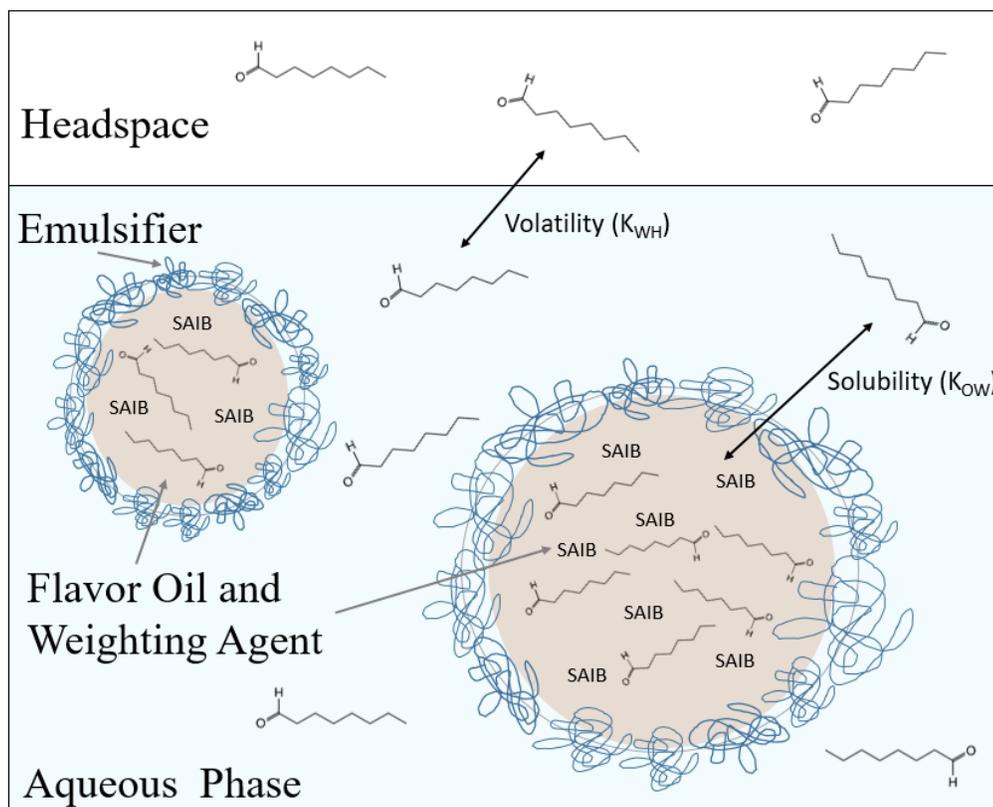


Figure 2.3. Schematic illustration of a beverage emulsion at a molecular size which contains a hydrophobic mixture of flavor oil and weighting agent (tan) entrapped by an emulsifier (blue lines) and dispersed within water (solid blue).

## CHAPTER 3: INTERACTIONS BETWEEN WEIGHTING AGENTS AND FLAVOR COMPOUNDS IN ESSENTIAL CITRUS OIL BLENDS

Kelsey Kanyuck, Chris Pernell, and Christopher Daubert

[to be submitted to *Journal of Food Science* with minor modifications]

### 3.1. Abstract

Flavor oil emulsions are widely used in citrus beverages, and weighting agents (WAs) are typically added to increase the oil phase density and inhibit emulsion instability. A shift in consumer acceptance of one WA, brominated vegetable oil (BVO), has prompted beverage manufacturers to reformulate products with alternative ingredients. Two such replacement WAs are sucrose acetate isobutyrate (SAIB) and ester gum (EG), but no published research has evaluated the implications of these agents on flavor. In this research, interactions between WAs and flavor oil components were characterized and modeled to build an understanding of the changes that accompany beverage reformulations. Orange, lemon, and lime oils blended with each WA were analyzed with headspace gas chromatography mass spectrometry (HS GC-MS) to measure changes in abundance of volatile flavor compounds. Clustering of flavor compounds and development of a linear model suggested oxygenated compounds are decreased in the headspace more than non-oxygenated compounds, with addition of a WA. A model was proposed to predict associations of flavor compounds to WAs considering polarity and polarizability of the molecule. Both the type of WA and the concentration affected the extent of flavor compound interactions. Descriptions of interactions between WA and flavor compounds will allow beverage companies to predict which compounds display the strongest association to WAs and thus require modification when substituting different concentration or types of WAs.

## 3.2. Introduction

Weighting agents (WAs) are used in beverage formulations to increase stability by decreasing the density difference between oil and water phases (Chanamai and McClements, 2000; McClements, 2016). The three FDA approved WAs in the United States are sucrose acetate isobutyrate (SAIB), brominated vegetable oil (BVO), and ester gum (EG) (21 C.F.R. § 172.833, 2015; 21 C.F.R. § 180.30, 1984; 21 C.F.R. § 172.735, 2007). BVO had been the most commonly used WA, but recent consumer concern over the safety of bromination has caused beverage manufacturers to evaluate replacement ingredients. Due to commercial availability, high density, and approval by the FDA, SAIB and EG have been considered as alternative WAs. Each of these three WAs has been demonstrated to effectively stabilize flavor emulsions, although different densities required different usage levels, (Chanamai and McClements, 2000), but the implications on flavor partitioning have not been addressed in published literature to date.

### 3.2.1. Background

The three WAs (SAIB, BVO, and EG) all have densities greater than water (1.0 g/mL) and are immiscible in water, but the chemistry of each molecule is quite different. Figure 2.2 compares the average molecular structure in each of the WAs. SAIB is a sucrose molecule octa-esterified with acetate and six isobutyrate moieties. BVO is a triglyceride, saturated with 4-6 bromine atoms on each fatty acid. EG is a mixture of wood rosin components, such as abietic acid, esterified to a glycerol backbone. Due to regulations, the maximum usage levels for each of the WAs is different, and the maximum and common usage levels are displayed in Table 2.1. These differences in usage ratios may impact flavor

compound partitioning due to shifts in molar ratios (Raoult's Law), in addition to any chemical differences between the WAs.

Citrus flavor oils typically require the usage of WAs for applications in beverages due to the low density and immiscibility in water of the oil. Over 200 compounds have been identified in essential orange oil, and 20-30 of these compounds are likely contributing to the characteristic orange flavor (Hinterholzer and Schieberle 1998; Buettner and Schieberle, 2001; Qiao et al. 2008; Xiao et al. 2016). Orange, lemon, and lime flavors are composed of primarily the same compounds in differing ratios, thus the distinctions between flavors are produced by slight variations in composition (Shaw, 1979; Perez-Cacho and Rouseff, 2008). The most prevalent classes of compounds in citrus oils are terpenes, saturated aldehydes, alcohols, and aldehydes (Shaw, 1979). Compounds analyzed in this experiment, and each corresponding chemical class, are listed in Table 3.2. Most of these compounds have been previously analyzed in orange oil flavor research (Mirhosseini et al. 2007; Mirhosseini and Tan, 2009; Mirhosseini et al. 2010). Chemical differences between compounds may create variation in interactions with WAs, and therefore a multivariate approach is useful to describe the impacts of WAs on multiple flavor compounds.

### 3.2.2. Measurement of Flavor-Ingredient Interactions

Due to variation in abundance and activity of the many molecules comprising flavors, approaches to normalize and compare flavor compounds have often included partition coefficients and quantitative structure activity relationships (QSAR) (Landy et al. 2000). The complex formation constant ( $K_f$ ) is a modified partition coefficient which has been used to compare the association of flavor compounds with specific analytes of interest (Astray et al. 2010; Landy et al. 2000). After adjustment of the models proposed by Astray et al. (2010)

and Rekharsky and Inoue (1998), Eq. 2.5 was developed to calculate a  $K_f$  for associations between WAs and flavor compounds. A common application for  $K_f$  in foods is the measurement of association between cyclodextrins and flavors in encapsulations (Landy et al. 2000; Astray et al. 2010; Reineccius et al 2005). Further steps to understand influences of each of the calculated  $K_f$  usually has included a type of QSAR analysis, like clustering or multiple linear regression (MLR). Clustering is a type of data reduction technique that groups samples by highest correlation and summarizes the greatest differences and similarities within a large data set. For this application, MLR uses molecular properties as parameters to form a model using linear combinations of parameters that predict the  $K_f$  of each molecule. A measure of molecular hydrophobicity, LogP, has been a commonly applied parameter to predict compound interactions (Charles et al. 2000; Carey et al. 2002; van Ruth et al. 2002; Reineccius et al. 2005; Mirhosseini and Tan 2009; Astray et al. 2010; Savary et al. 2014). The molar refractivity (A) describes the polarizability of the molecule. Other parameters used in QSAR have included values that reflect size, orientation, volatility, or surface structure of a molecule. Many parameters are not correlated to the response, but significant parameters in the final models are suggested to be the driving factors of the interaction because of the correlation with the estimated variable (Astray et al. 2010). These multivariate analyses have built further understanding and generalizations of compound interactions, allowing simplified application of results, and both of which were applied in this experiment.

In other food systems, ingredient modifications have been demonstrated to have an impact on compound partitioning (Reineccius et al. 2005; Chang, 2012; Savary, 2010) and even flavor perception (Hatchwell, 1996; Deibler and Acree, 2000; Charles et al. 2000). Changes in the partitioning of flavor compounds represent an interaction or association with

the altered ingredient (Overbosch et al. 1991). Increasing the oil phase volume has been demonstrated to shift the partitioning of flavor compounds towards a greater number of hydrophobic compounds in the oil phase (Malone et al. 2000; Leksrisonpong et al. 2010). These changes in partition coefficient were hypothesized to be caused by the greater entropy within the oil phase, from the mixing of multiple hydrophobic compounds (McClements et al. 2012). Chemical natures of these compounds were also shown to influence the interactions, since not all flavor compounds displayed the same changes (Donsi et al. 2012; Leksrisonpong et al. 2010) and not all additives produced equivalent changes (Schober and Peterson, 2004; Taylor et al. 2009). Many systems have displayed changes with addition of hydrophobic additives, suggesting that WAs are likely to also impact flavor molecule partitioning from flavor oils.

### 3.2.3. Research Objectives

The objectives of this research were to analyze the effects of WAs in flavor oil blends and to model these effects. Past research has suggested that the chemistry of flavor compounds influences their interactions. Thus, the focus of this work was to examine the many flavor compounds comprising citrus oils and separately assess their interactions. To model these behaviors, three WAs (SAIB, BVO, and EG) were each dissolved in three flavor oils (orange, lemon, and lime), and volatile components of these flavor oils were measured in the presence and absence of each individual WA. After normalization, multivariate statistics were applied to the flavor compounds for each WA and flavor oil to produce a generic generalization of the interactions. The resulting model will be applied to help explain the changes between WAs and suggest a method to overcome these differences.

### 3.3. Materials and Methods

#### 3.3.1. Sample Preparation

Oil blends were prepared by dissolving each WA (SAIB, EG, BVO) at 0.04, 0.32, 0.52, and 1.0 molal into each flavor oil. The three flavor oils ((5x essential orange, 5x essential lemon, and washed lime oil) were purchased from Berjé Inc. (Carteret, NJ). The SAIB and EG were acquired from Eastman Chemical Company (Kingsport, TN), and the BVO was purchased from Spectrum Chemical Manufacturing Corp. (New Brunswick, NJ). The molality (moles of solute/kg of solvent) of WA in oil blends were calculated by assuming the WAs were pure mixtures of each predominate component (SAIB: a sucrose diacetate and hexaisobutyrate at 846.9 g/mol, EG: a triglyceride with three abietic acid moieties at 917.4 g/mol, and BVO: a triglyceride with one fatty acid linoleic acid derivative with four brominated carbons and two oleic acid derivatives with two brominated carbons at 1550.7 g/mol). Usage ratios are defined as 50 ppm SAIB, 8 ppm BVO, and 50 ppm EG in the final beverage formulation which corresponds to 0.52 m, 0.04 m, and 0.52 m solutions respectively with a constant oil mass of 0.01%. Samples were prepared in triplicate by adding each WA to a glass vial of flavor oil and equilibrating, while shaking at 1.0 rpm, for at least 72 hours at 25°C to fully dissolve. For spiked samples, analytical standards for octanal and citronellal (Sigma Aldrich, St. Louis, MO) were diluted in orange oil to create a stock solution. These stock solutions were separately added to a concentrated blend of SAIB in orange oil to reach a final level of 0.52 m SAIB. Each spiked sample was created and run in triplicate.

### 3.3.2. Instrumental Analysis

*Chromatography:* The GC-MS analysis was carried out using a 7820A GC and 5977E quadrupole MSD (Agilent Technologies, Santa Clara, USA). Sample volumes of 50.0  $\mu$ L of each prepared oil were added to a 20 mL glass headspace vial and heated to 50  $^{\circ}$ C, with agitation, for 20 minutes. Using a CTC CombiPAL autosampler (Lake Elmo, MN), 1.0 mL of headspace was injected with a 1:10 split at an inlet temperature of 275  $^{\circ}$ C. Helium gas was used as the mobile phase at a flow rate of 1.2 mL/min on a J&W HP-5 MS column (30 m $\times$ 0.25 mm I.D, 0.25  $\mu$ m film thickness (Agilent Technologies, Santa Clara, USA)). The temperature ramp of the oven started with a 3 minute hold at 40  $^{\circ}$ C, increased at 3 $^{\circ}$ C/min until 115 $^{\circ}$ C with a 1 minute hold at 71 $^{\circ}$ C, and increased at 45 $^{\circ}$ C/min until 250 $^{\circ}$ C with a 1 minute hold. The source temperature of the MSD was set at 230  $^{\circ}$ C, the quadrupole temperature at 150  $^{\circ}$ C, and mass spectra were collected with a range of ions from 30–250 m/z. Compounds were tentatively identified by comparing the MS fragmentations and retention index (RI) from each peak to literature values (Adams, 1989; Qiao et al. 2008). The identities of limonene,  $\beta$ -pinene, linalool, hexanal, octanal and nonanal were confirmed with pure compound standards (Sigma Aldrich, St. Louis, MO).

*Densitometry:* Using a Mettler Toledo DE40 Density Meter (Columbus, OH), the density of each flavor oil blend was measured at 23  $^{\circ}$ C. Approximately 1.5mL of liquid was added to the instrument for each measurement, and the tubes were cleaned with water, acetone, and air dried between each sample.

*Rheometry:* The apparent viscosity of each flavor oil blend was measured with an Anton Paar MCR 302 Rheometer (Ashland, VA) equipped with a double walled cup and bob

attachment. Analysis was conducted with shear rates from 0.1 – 100 s<sup>-1</sup> at 10 °C to mimic refrigeration temperatures.

### 3.3.3. Volatile Compounds Calculations

The peak area of each tentatively identified compound was compared to the control (no WA) of each flavor oil to calculate the relative headspace intensity (RHI):

$$\text{RHI} = \frac{\text{Peak area of sample}}{\text{Peak area of control}} \times 100 \quad [\text{Eq 3.1}]$$

The RHI was calculated for each concentration of WA in each flavor oil to normalize the naturally wide range of abundance and peak areas of compounds in flavor oils. A similar normalization procedure was previously used by Carey et al. (2002) to calculate a RHI with volatile headspace measurements in flavor emulsions. To account for the influence of WA molality, the complex formation constant ( $K_f$ ) was calculated (Eq 2.5) for each flavor compound. After normalizing inherent differences between flavor compounds, multivariate statistics were applied to summarize WA interactions.

### 3.3.4. Data Analysis and Modeling

Statistical clustering (ProcCluster) using SAS 9.4.2 Software (Cary, NC) was used to group similar volatile compounds by their variance in RHI across three WAs at two concentrations. The centroid method was specified, and the number of clusters was restricted to four. The analysis was performed on the orange oil samples, and the clusters were then applied to the lemon and lime samples. Multiple linear regression (MLR) modeling was used to predict the  $K_f$  of each flavor compound based on a variety of molecular parameters. The molecular properties used as possible parameters in the model were: the octanol-water

partition coefficient ( $\log P$ ), molar refractivity ( $A$ ), topological polar surface area (TPSA), molar volume, TPSA/molar volume, polarizability, partition coefficients between octanol and air ( $\text{Log}K_{oa}$ ), air and water ( $\text{Log}K_{aw}$ ), and octanol and water ( $\text{Log}K_{ow}$ ), number of rings, and the squares and products of those listed. These molecular properties were assembled from estimates by Molinspiration Cheminformatics ([www.molinspiration.com](http://www.molinspiration.com)) and ACD/Labs Percepta Predictors - PhysChem Module (available through [ChemSpider.com](http://ChemSpider.com)). The software used for modeling was SAS 9.4.2 (Cary, NC) and the final model utilized forward selection to account for the most variance (by the highest  $R^2$ ) with the least degree of complexity.

### **3.4. Results and Discussion**

The implications of both the WA type and concentration were evaluated in citrus flavor oils. Because each WA has a different density (Table 3.1), different concentrations of WA must be added to an oil to achieve the desired density for beverage flavor application. This study was designed to consider molecular interactions and associations between WAs and flavor compounds at typical usage levels in beverage flavor oils, and did not aim to measure interactions or flavor changes in an actual beverage product.

#### **3.4.1. Physical Properties of Flavor Blends**

The physical properties of flavor oil blends are important to consider during beverage formulation with respect to the necessary usage levels and processing methods (Tan, 2004). Density and viscosity were measured on unweighted flavor oils, as well as the weighted oils at typical usage ratios (0.52m SAIB, 0.04m BVO, and 0.52m EG, see Table 3.1). The addition of any of the WAs increased the density of the flavor oil (originally 0.862 for orange oil), and all materials displayed Newtonian behavior over the selected range of shear rates. At

application usage levels, EG increased the oil blend viscosity the greatest, and the BVO increased the oil blend viscosity the least. Although all blends remained in the liquid state at usage rates, the differences in viscosity could impact processing considerations of the flavor emulsion (homogenization efficiency) and final beverage or rates of flavor release. The density of each blend at usage ratios was also measured, and results are shared in Table 3.1. In the original form, BVO had the highest density, followed by SAIB and then EG with the lowest. These density differences require different usage ratios to achieve the same final oil blend density. Based on common usage ratios, each type of weighted blend achieves a different final density (Table 3.1). To match the same density, BVO would need to be added at lower levels, and EG would need to be added at the highest level. Maximum levels in final beverages have been set by the FDA and must also be considered during formulation (Table 2.1). These differences in concentrations may impact the type or extent of interactions with flavor compounds in the oil blends.

#### 3.4.2. Implications of WA Concentration

The effects of WA presence and concentration on the volatility of flavor compounds from orange, lemon, and lime oils were measured. The RHIs for each compound and WA blend are reported as the average of three replicates in Table B.1. The headspace above these flavor oil blends can be considered a saturated equilibrium, and as such, the headspace concentration is a function of the relative partial pressures of each compound and not their direct concentration in the sample, as with most food matrices (Kolb and Ettre, 2006). The measured relative headspace intensities (RHIs) in this research were therefore not a true equilibrium value, but instead a measure of binding affinity that considered other compounds within the system, which also influenced complex formation. Interactions between flavor

compounds, stronger affinities of certain compounds, and a 'saturation' of WA surface all shifted these measurements from true RHI and  $K_f$  values. Instead, a relative binding affinity was expressed for each of the flavor compounds which included these factors. Since flavor oils are commonly employed ingredients in beverage application, this research represented a more realistic picture of the complex interactions within mixtures such as molecular competition for binding sites and associative interactions.

With increasing concentration of any of the WAs, a volatility decrease was observed for flavor compounds. Changes in RHI of two selected flavor molecules (linalool and  $\beta$ -pinene) are shown in Figure 3.1. At 0.04 m SAIB and 0.04 m BVO, there was a significant decrease in the volatility of linalool from the unweighted sample. This decrease in volatility with increasing nonpolar additive was likely due to changes in mole fractions or entropy and has been observed previously with other solvents in emulsions (Harrison et al. 1997; Carey et al. 2002; van Ruth et al. 2002; Malone et al. 2000; Leksrisonpong et al. 2010). Chang et al. (2012) observed that higher amounts of additive in the lipid phase decreased the antimicrobial efficiency of a thyme oil (assumed to be correlated with the solubility of the active agents in the oil), but the two tested oils did not have equal impacts. From a thermodynamic equilibrium perspective, the hydrophobic additive was hypothesized to decrease the flavor compounds entropy of mixing, caused by the inherent greater entropy and enthalpy within the oil phase with the additive than without (Chang et al. 2012). Consistent with previous studies, adding material to the oil phase (in this case WAs) had an impact on the partition coefficients of the active agents. These experiments suggested a stoichiometric importance of WA on the partition coefficients of flavor compounds.

Furthermore, for any single flavor molecule, differences between WAs suggested volatility changes were not on a pure molality relationship. The volatility of linalool with 0.52 m SAIB was 66% RHI, while BVO at 0.52m resulted in 83% RHI. These differences suggested a specific association, or type of association, between WAs and certain types of flavor compounds. Due to differences in density among the WAs, each must be used at different concentrations to reach the same oil phase density, and this concentration adjustment was observed to further increase the difference between SAIB and BVO. For example, at typical usage ratios of SAIB (0.52 m) and BVO (0.04 m), the RHI of linalool was significantly different at 66% and 91% respectively ( $p < 0.05$ ). Therefore, this requisite difference in usage ratios that modified partitioning of volatile compounds may contribute to differences in flavor in beverage applications.

#### 3.4.3. Influences of Flavor Compound Structures

Although WA concentration was suggested to impact flavor volatility, the effect was not the same for all compounds. As seen with the comparison between linalool and  $\beta$ -pinene, flavor compounds were not affected equally by WAs (Figure 3.1). For the same molality of SAIB, linalool displayed a volatility of 66% RHI, while  $\beta$ -pinene showed an 88% RHI. The two compounds, linalool and  $\beta$ -pinene, (Figure 3.1) represented a small piece of the story, and all compounds should be considered for complete characterization of WA influences. To summarize the effects of WAs on all flavor compounds, the RHI of SAIB was plotted against BVO, with each dot representing a single flavor compound (Figure 3.2A). The line of equality signifies equal interaction between each of the two WAs (grey dashed line), and the star signifies no change from control (100% RHI). Some molecules had a RHI of 50% (half of the initial concentration), while others appeared to have little or no change in the

headspace intensity (Figure 3.2A). Variability of WA impacts on flavor compounds was emphasized by the spread of RHI of each analyzed flavor compound (Table 3.2). Statistical clustering was used to group similar compounds by RHI with SAIB, BVO, and EG. This grouping organized compounds by molecular structure within clusters and were named as such (terpenes, alcohols, saturated aldehydes, and terpenoids). This grouping of compounds into clusters is shown in Figure 3.2B. Each circle is centered at the average of the cluster, and the size is relative to the standard deviation within the cluster. The cluster compositions are shown in Table 3.2 and average values summarized in Table 3.3. The comparison of EG and BVO at typical usage levels is shown in Figure 3.3.

The clustering of compounds by their chemical class suggests the chemistry of a compound impacts the magnitude of WA influence. Oxygenated molecules (aldehyde, alcohols, and terpenoids) showed more associations to WAs than terpene molecules. The functional groups on these molecules (alcohols, aldehydes, and double bonds) are therefore suggested to be a controlling factor of the associations with WAs. Preferential association of some flavor molecules to different additives have been reported in cyclodextrin research (Reineccius et al. 2005; Astray et al. 2010) and emulsions (Savary et al. 2014). The associations were linked to specific molecular attributes, suggested to be important due to the size and hydrophobicity of the binding pocket, which selected for these types of compounds (Astray et al. 2010). Driving principles of the interaction were predicted through the determination of important molecular attributes. Due to the clustering of flavor compounds by their chemical structure, the WAs appeared to form associations with the oxygenated functional groups.

#### 3.4.4. Effects of Flavor Oil Composition

In each flavor oil, cluster interactions with WAs appear to stay consistent. The terpenoid compounds (blue, Figure 3.4) were the most impacted molecules in each of the three flavor oils. Similarly, the terpene compounds (green, Figure 3.4) were the least impacted, as demonstrated by the average near to 100% of the control (or 100% RHI). Consistency of compounds in each cluster, and the relative magnitude of associations, suggest a universality of interactions by compound class. Although the clustering is similar between flavor oils, the magnitude of these changes is not. Instead, it appears that the type of flavor influences the magnitude of changes, even when equal amounts of WA were used.

When comparing RHI of various flavor oils, it appeared that the composition of the flavor oil affects the magnitude of WA influence. The terpenoid cluster in orange oil had an average RHI with SAIB of 46% while in lemon it was 79% and in lime 75% (Appendix 1). Since these flavor oils are primarily comprised of the same compounds in different ratios, the ratios of these compounds were suggested to influence the extent of changes with substitutions of WAs. In each flavor oil, the terpenoid cluster was comprised of citronellal, neral, and geranial, but the amount of these compounds in the control was different. In orange oil, the summation of peak areas for terpenoids was  $57.6 \times 10^3$ , lemon was  $928.0 \times 10^3$ , and lime was  $9.2 \times 10^3$ . In the case of orange oil, the relative abundance of terpenes (specifically limonene) is higher than lime or lemon oil (Shaw, 1979), thus the abundance of oxygenated compounds is lower, and this difference in amount appears to have caused the WAs to display a larger impact. An explanation for these inconsistencies between flavor oils may be differences in the relative number of oxygenated compounds (molecules with an oxygen moiety such as terpenoids, aldehydes, and alcohols) which are present. Since only

oxygenated compounds were suggested to associate with WAs, the amount of these compounds in a flavor is critical to assessing and predicting the influence of a WA. The non-oxygenated compounds (terpenes) displayed minimal association to WAs and are presumed to not bind with a WA. Flavor blends with a low abundance of oxygenated compounds (such as the orange flavor oil) would be more likely to show a difference when switching between WAs due to the higher ratio of WA to analyte. Flavors with a large abundance of oxygenated flavor molecules are consequently less likely to display differences with WAs since the effect of WA is spread over many different compounds. In a simplified generalization, when 100 molecules of WA are added, 100 molecules of oxygenated flavor compounds would associate and not volatilize. When there are only 400 molecules of oxygenated flavor compounds in the flavor, 25% of the molecules are excluded from volatilization; alternatively, if 4000 oxygenated flavor compounds are present, the impact drops to only 2.5%, and this change is less likely to be detectable. For a flavor oil with few oxygenated compounds present, it is hypothesized that adding or switching the WAs will have profound impact.

#### 3.4.5. Predicting the Influences of Flavor Compound Structures

Based on the previously discussed factors, the structure of each flavor compound is suggested to dictate the extent of interaction with a WA. For a more detailed prediction than the previously discussed clustering, the technique of QSAR was applied to model the interactions by the molecular properties of flavor compounds. For each compound, a  $K_f$  was calculated based on the interaction with each WA using HS GC-MS and Eq 2.5. The compounds identified in the flavor oils which were used for analysis, including their structures, chemical classes, logP values, and A values, are shown in Table 3.2. Many

chemical and physical parameters, as discussed in the methods section, were included as parameters in building a MLR equation to estimate the  $K_f$ . All molecular properties utilized in the linear regression are displayed in Table B.2 and correlation between variables is shown in Table B.3. The best-fit equation, with an  $R^2$  of 0.86, for the  $K_f$  between SAIB and orange oil compounds was:

$$K_f = 0.6 - 0.24*\text{LogP} + 0.013*A \quad [\text{Eq 3.2}]$$

Based on this equation, differences in the molecular variables logP and A for each flavor compound can account for 86% of the variability within the data set. The correlation between the predicted  $K_f$  using LogP and A and the measured  $K_f$  is shown in Figure 3.5A. To test the adequacy of the model, the experiment was repeated with new flavor oil blends which were not in the original model (Figure 3.5B). The predicted equation 3.2 was applied to the new data set, and was suggested to be an appropriate model with an  $R^2$  of 0.71. This analysis did not attempt to apply meaning to the numerical constants in the equation, but merely which variables are driving forces for the interaction. However, based on the contribution of each property to the  $K_f$  prediction, the chemical basis of the association can be estimated.

Other researchers have also utilized MLR to model the effects of various flavor compounds with their molecular properties (Landy et al. 2000; Carey et al. 2002). Astray et al. (2010) used a similar approach to predict the  $K_f$  of flavor compounds to cyclodextrins, and the two molecular parameters in the final model were determined to be the driving factors of the association. In cyclodextrins, the logP and molecular size were most correlated to the  $K_f$  of flavor compounds (Astray et al. 2010). The molecular parameters (size and logP) were explained by the size of the cone-shaped binding pocket and hydrophobic interior of the

cyclodextrin molecule (Astray et al. 2010). In each case, the significant variables included in the linear model were hypothesized to be the driving factors of the measured change.

The two parameters included in Eq. 3.2 suggested the driving forces for the WA-flavor compound interaction were reflected in those molecular properties. Accordingly, the hydrophobicity or polarity (measured by the log P) and polarizability (measured by A) were suggested to be important contributors to the WA interaction. For differentiation, polarity describes the constant dipole within a molecule, while the polarizability describes the degree to which a molecule can be induced to have a dipole within an electric field (Israelachvili, 2011). The negative sign of the logP component in the equation suggested a more hydrophobic molecule will have a lower association with SAIB, and the positive value with A suggested a more polarizable molecule will have higher associations. The importance of polarity and polarizability supports the presence of both Van der Waals dispersion forces and anti-hydrophobic interactions are responsible for the WA associations. Molecular associations are typically caused by the combination of several weak interaction mechanisms (Rekharsky and Inoue, 1998). Therefore, due to the two significant molecular properties, WA associations were hypothesized to form through several dispersive interactions occurring within a relatively hydrophilic region of the WA. In a more generalized interpretation, the addition of WAs increases the dispersive interactions in the solution and unequally decreases the flavor compounds more prone to dispersive interactions.

The hydrophobicity and polarizability factors of associations with WAs may help explain the clustering by chemical class observed in Figure 2. Terpenoid molecules (blue cluster) have the lowest hydrophobicity and the highest polarizability, both of which were suggested to increase WA interactions through Eq. 3.2. Alternatively, the terpene molecules

(green cluster) have the highest hydrophobicity and lowest polarizability, which both predict a low association per Eq 3.2. Due to the similarity in  $A$  for the terpene molecules, this parameter only contributes to the  $K_f$  prediction for the terpenoid, alcohol, and saturated aldehyde flavor compounds, suggesting the molecular polarizability is only important in distinguishing oxygenated flavor compounds. This MLR equation (Eq. 3.2) predicted that interactions were dependent on the molecular properties of the flavor compound, which helps to explain why flavor compounds were variably impacted by WAs.

#### 3.4.6. Adjustments to Account for WA Differences

The changes in concentration and type of WA contributed to modifications in flavor compound volatility. Thus, methods to overcome changes in WA without modifying the flavor perception would be of value to beverage manufacturers. While the WA concentration is governed by the flavor oil density, and therefore cannot be easily altered, it is feasible to modify the flavor oil composition. A method of adding pure compounds to flavor oil blends (spiking) was proposed and evaluated to mitigate the impacts of WA adjustments.

The spiking of pure compounds into flavor oils blends was proposed to overcome the changes in volatility of highly impacted (terpenoid, alcohol, and aldehyde) compounds. The spiking method was demonstrated to increase the RHI of octanal and citronellal in blends with SAIB to be closer to the respective RHI with BVO as the WA. The spiking of 100ppm octanal into a 0.52 m SAIB orange oil blend increased the RHI closer to the 0.04 m BVO RHI of octanal, while the other compounds were not significantly different (Figure 3.6A). Similarly, the same trend of increasing the RHI of the selected compound observed with the spiking of 25 ppm citronellal (Figure 3.6B). This technique was applied as a proof-of-concept to suggest adding pure compounds to a flavor blend can overcome the inhibitory

effects of the WAs on volatility of these flavor compounds. Compounds with a lower logP or higher A, as described by the linear model, should be added to existing flavor oil blends, relative to their predicted  $K_f$  values, to overcome the changes when switching WAs.

### **3.5. Conclusion**

This work demonstrated the influences of WAs on flavor compounds within citrus oils, and a model was proposed to estimate the magnitude of influence based on chemical properties of each compound. Further, compound spiking was proposed to account for different WAs and different usage ratios, and success was demonstrated with two compounds. The addition of a WA decreased the availability of certain types of flavor compounds, and the amount of WA was suggested to be important. Due to inherent chemical and usage level differences, switching between WAs changed the volatile flavor compound profile. Thus, strategies to offset changes are required for switching between WAs, and compound spiking was demonstrated to be a viable solution. Future research should address a broader range of molecules with more diverse molecular properties (such as size and logP) to confirm the applicability of the hypothesized phenomenon to the wide range of flavor compounds used in beverages. Although model systems were used in this research, beverage manufacturers are recommended to use the proposed model to guide their reformulations as a suggestion of which types of compounds are most affected by WA changes.

### 3.6. References

- Adams RP. 1995. Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, Ill. : Allured Pub. Corp., viii, 469 pages : illustrations ; 24 cm p.
- Astray G, Mejuto JC, Morales J, Rial-Otero R, Simal-Gándara J. 2010. Factors controlling flavors binding constants to cyclodextrins and their applications in foods. *Food Res.Int.* 43(4):1212-1218.
- Buettner A, Schieberle P. 2001. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J.Agric.Food Chem.* 49(5):2387-94.
- Carey ME, Asquith T, Linforth RS, Taylor AJ. 2002. Modeling the partition of volatile aroma compounds from a cloud emulsion. *J.Agric.Food Chem.* 50(7):1985-90.
- Chanamai R, McClements DJ. 2000. Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J.Agric.Food Chem.* 48(11):5561-5.
- Chang Y, McLandsborough L, McClements DJ. 2012. Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening inhibitors. *J.Agric.Food Chem.* 60(48):12056-63.
- Charles M, Lambert S, Brondeur P, Courthaudon J, Guichard E. 2000. Interactions of flavor compounds with food components - 28 Influence of Formulation and Structure of an Oil-in-Water Emulsion on Flavor Release. ACS symposium series. 763342.
- Deibler KD, Acree TE. 2000. Effect of Beverage Base Conditions on Flavor Release. ACS Symp.Ser. 763333-341.
- Donsì F, Annunziata M, Vincensi M, Ferrari G. 2012. Design of nanoemulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. *J.Biotechnol.* 159(4):342-350.
- Harrison M, Hills BP, Bakker J, Clothier T. 1997. Mathematical Models of Flavor Release from Liquid Emulsions. *Journal of Food Science -Chicago-* 62(4):653-658.
- Hatchwell LC. 1996. Implications of Fat on Flavor. ACS symposium series. 63314.
- Hinterholzer A, Schieberle P. 1998. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragrance J.* 13(1):49-55.

Israelachvili JN. 2011. Intermolecular and Surface Forces: Revised Third Edition (3). US: Academic Press, 2011. ProQuest ebrary. Web. 8 November 2016.

Kolb B. 2006. Static headspace-gas chromatography : theory and practice. Hoboken, N.J.: Wiley-Interscience.

Landy D, Fourmentin S, Salome M, Surpateanu G. 2000. Analytical Improvement in Measuring Formation Constants of Inclusion Complexes between  $\beta$ -Cyclodextrin and Phenolic Compounds. *Journal of inclusion phenomena and macrocyclic chemistry* 38(1-4):187-198.

Leksrisonpong PP, Barbano DM, Foegeding AE, Gerard P, Drake MA. 2010. The Roles Of Fat And Ph On The Detection Thresholds And Partition Coefficients Of Three Compounds: Diacetyl,  $\Delta$ -Decalactone And Furanol. *J.Sens.Stud.* 25(3):347-370.

Malone ME, Appelqvist IAM, Goff TC, Homan JE, Wilkins JPG. 2000. A Novel Approach to the Selective Control of Lipophilic Flavor Release in Low Fat Foods. *ACS Symp.Ser.* 763212-229.

McClements DJ, Henson L, Popplewell LM, Decker EA, Jun Choi S. 2012. Inhibition of Ostwald Ripening in Model Beverage Emulsions by Addition of Poorly Water Soluble Triglyceride Oils. *J.Food Sci.* 77(1):C33-C38.

McClements DJ. 2016. *Food Emulsions : principles, practice, and techniques.* 3rd ed. Boca Raton : CRC Press, Taylor & Francis Group.

Mirhosseini H, Salmah Y, Nazimah SAH, Tan CP. 2007. Solid-phase microextraction for headspace analysis of key volatile compounds in orange beverage emulsion. *Food Chem.* 105(4):1659-70.

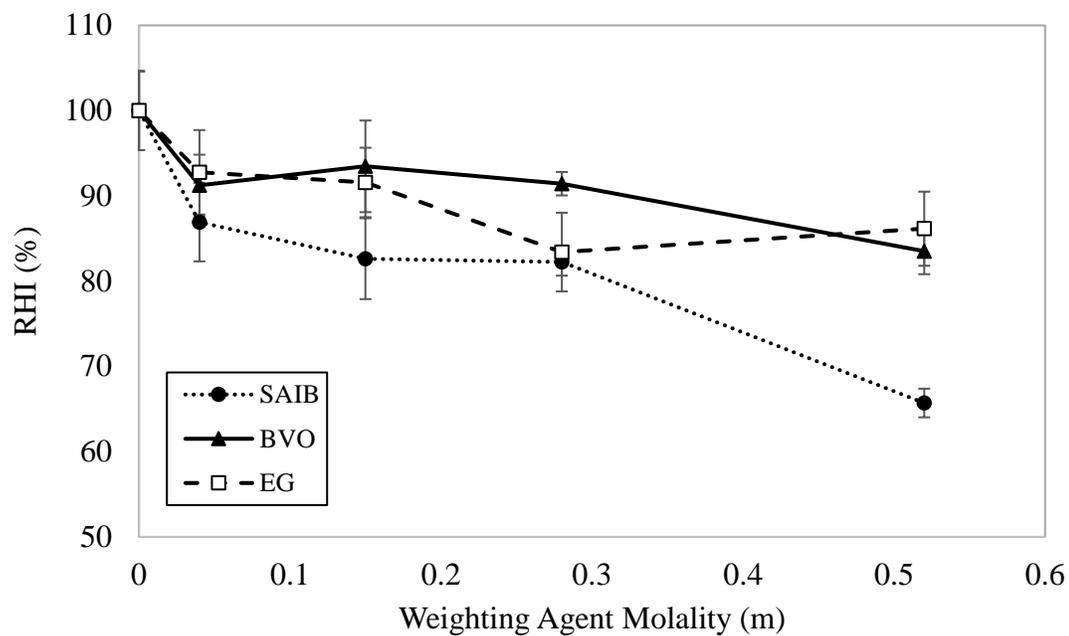
Mirhosseini H, Tan CP. 2009. Response surface methodology and multivariate analysis of equilibrium headspace concentration of orange beverage emulsion as function of emulsion composition and structure. *Food Chem.* 115(1):324-333.

Mirhosseini H, Tan CP, Kostadinovic S, Naghshineh M. 2010. Principle component analysis of equilibrium headspace concentration of beverage emulsion as function of main emulsion components. *J Food, Agriculture & Environment* 8(2):126-133.

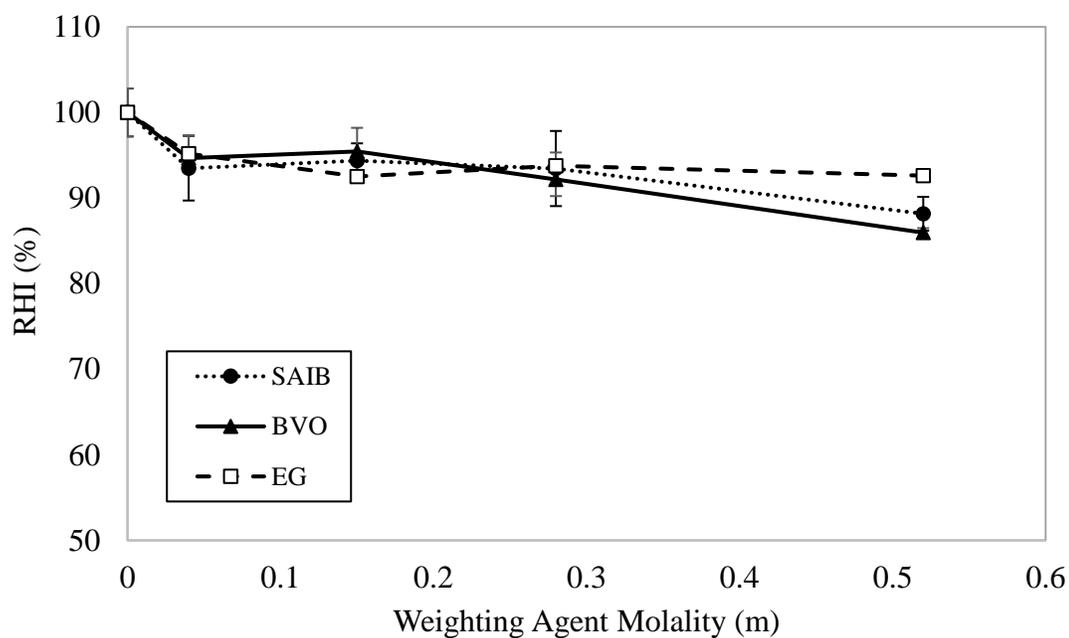
Overbosch P, Afterof WGM, Haring PGM. 1991. Flavor release in the mouth. *Food Rev.Int.* 7(2):137-184.

Perez-Cacho PR, Rouseff RL. 2008. Fresh squeezed orange juice odor: a review. *Crit.Rev.Food Sci.Nutr.* 48(7):681-95.

- Qiao Y, Xie BJ, Zhang Y, Fan G, Yao XL, Pan SY. 2008. Characterization of aroma active compounds in fruit juice and peel oil of Jincheng sweet orange fruit (*Citrus sinensis* (L.) Osbeck) by GC-MS and GC-O. *Molecules* (Basel, Switzerland) 13(6):1333-44.
- Reineccius TA, Reineccius GA, Peppard TL. 2005. The effect of solvent interactions on alpha-, beta-, and gamma-cyclodextrin/food molecular inclusion complexes. *J.Agric.Food Chem.* 53(2):388-92.
- Rekharsky MV, Inoue Y. 1998. Complexation Thermodynamics of Cyclodextrins. *Chem.Rev.* 98(5):1875-1918.
- Savary G., Hucher N., Bernadi E., Grisel M., Malhiac C. 2010. Relationship between the emulsifying properties of Acacia gums and the retention and diffusion of aroma compounds. *Food Hydrocoll.* 24(2-3):178-183.
- Savary G, Hucher N, Petibon O, Grisel M. 2014. Study of interactions between aroma compounds and acacia gum using headspace measurements. *Food Hydrocoll.* 371-6.
- Schober AL, Peterson DG. 2004. Flavor release and perception in hard candy: influence of flavor compound-compound interactions. *J.Agric.Food Chem.* 52(9):2623-7.
- Shaw PE. 1979. Review of quantitative analyses of citrus essential oils. *J.Agric.Food Chem.* 27(2):246-257.
- Tan, C. 2004. Beverage Emulsions. In: Larsson, K., & Friberg, S. *Food emulsions*. 4th ed., rev. and expanded. New York: M. Dekker. 485-524.
- Taylor AJ, Pearson K, Hollowood TA, Linforth RST. 2009. Aroma Release at the Nano- and Microscale: Molecules to Droplets. *ACS Symp.Ser.* (1007):246-258.
- van Ruth SM, King C, Giannouli P. 2002. Influence of lipid fraction, emulsifier fraction, and mean particle diameter of oil-in-water emulsions on the release of 20 aroma compounds. *J.Agric.Food Chem.* 50(8):2365-71.
- Xiao Z, Ma S, Niu Y, Chen F, Yu D. 2016. Characterization of odour-active compounds of sweet orange essential oils of different regions by gas chromatography-mass spectrometry, gas chromatography-olfactometry and their correlation with sensory attributes. *Flavour Fragrance J.* 31(1):41-50.
- Code of Federal Regulations. Title 21, sec. 172.833. (accessed Dec 1, 2015).
- Code of Federal Regulations. Title 21, sec. 180.30. (accessed Dec 1, 2015).
- Code of Federal Regulations. Title 21, sec. 172.735. (accessed Dec 1, 2015).



(A)



(B)

Figure 3.1. Impacts of weighting agent molality on the volatility of two selected compounds in orange oil (A: linalool, an alcohol; B:  $\beta$ -pinene, a terpene). Error bars represent one standard deviation of each data point, which were run in triplicate.

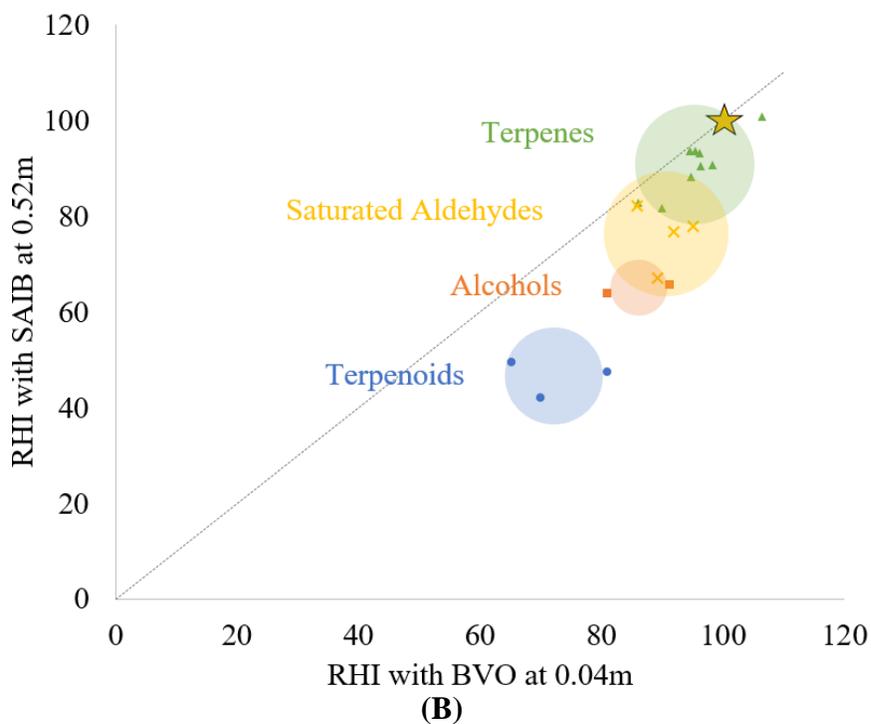
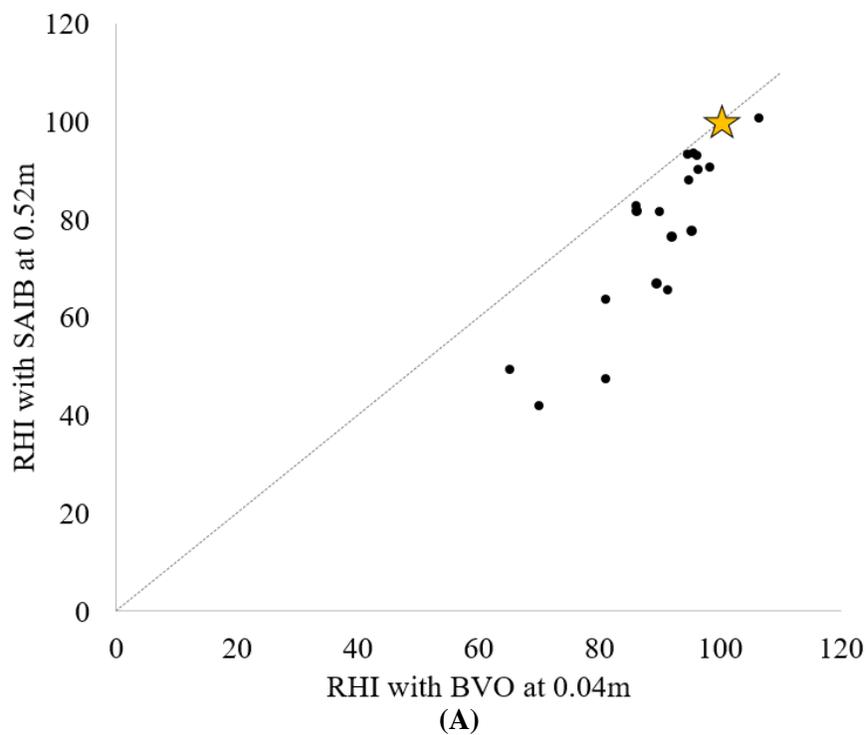


Figure 3.2. Variability in magnitudes of orange oil volatile compounds interactions with SAIB and BVO (A) at typical usage ratios (0.52m SAIB and 0.04m BVO) and (B) with overlays of clusters. The star represents 100% RHI or no change from control.

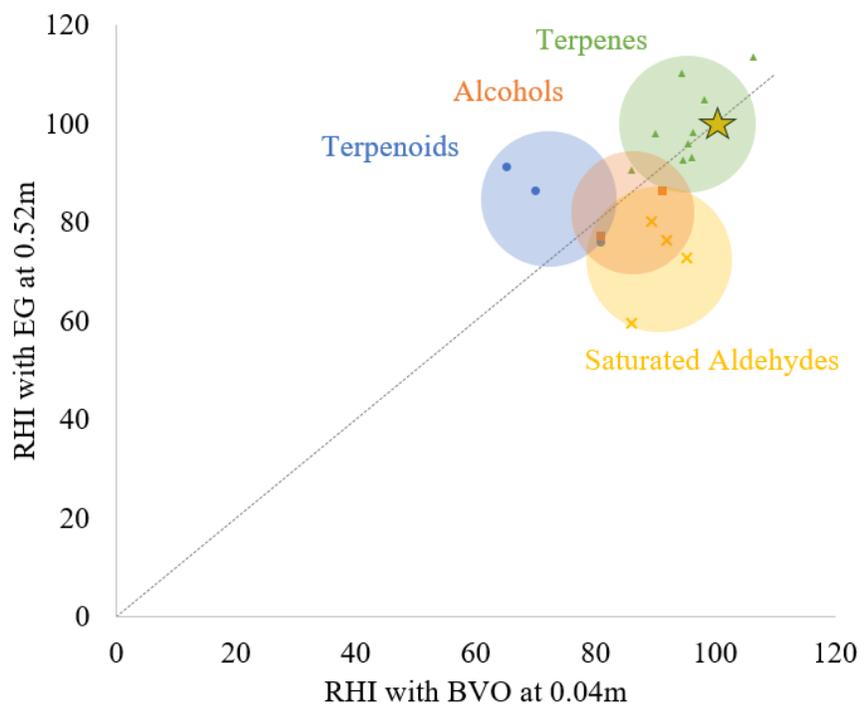


Figure 3.3. Variability in magnitudes of orange oil volatile compounds interactions with EG and BVO at typical usage ratios (0.52m EG and 0.04m BVO) with overlays of clusters. The star represents 100% RHI or no change from control.

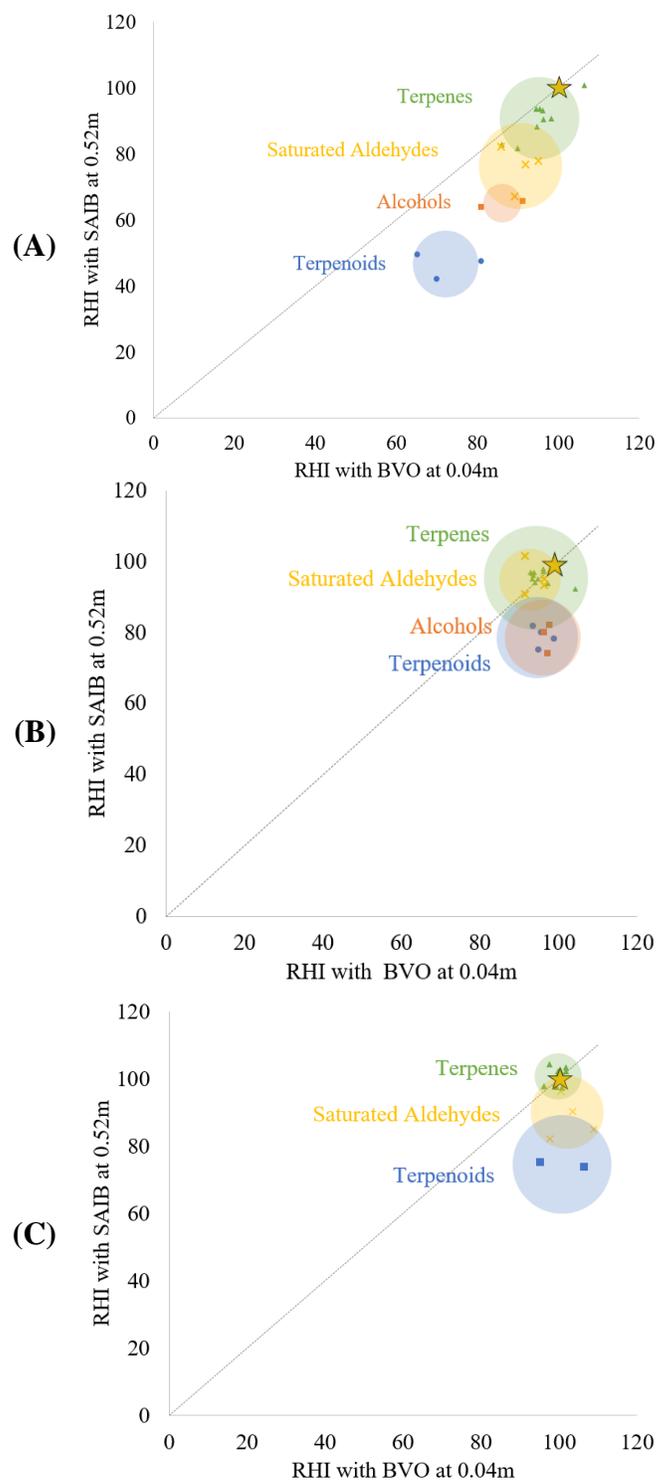
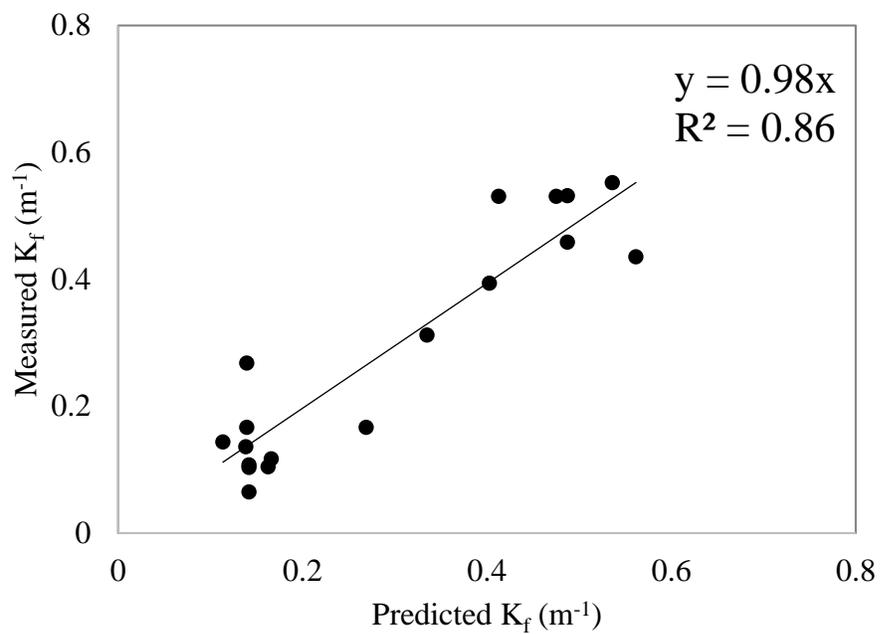
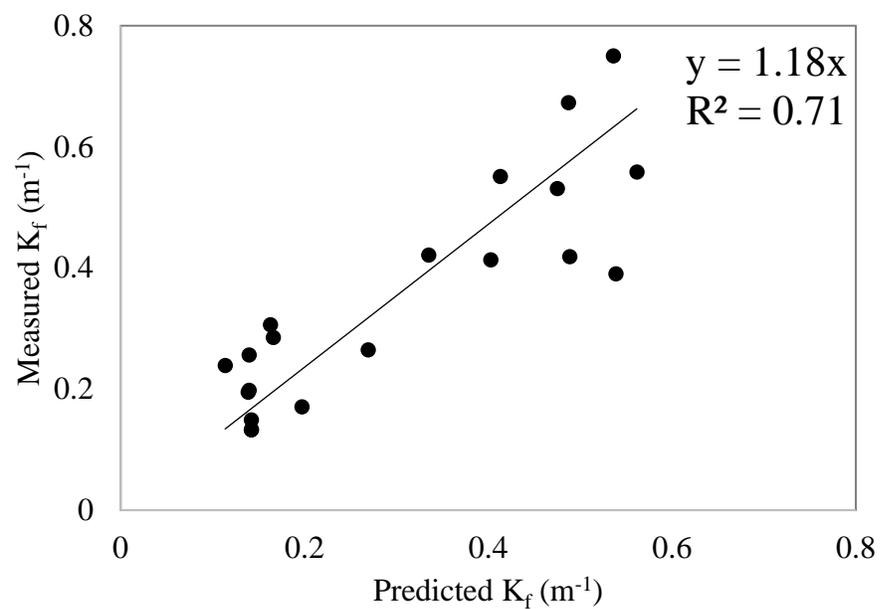


Figure 3.4. Comparison of the magnitude of (A) orange, (B) lemon, and (C) lime flavor oil components volatility changes with the weighting agents SAIB and BVO at usage ratios (0.52m SAIB and 0.04m BVO). The star represents 100% RHI or no change from control.



(A)



(B)

Figure 3.5. Quality of the linear model  $K_f(\text{SAIB}) = 0.6 - 0.24 \cdot \text{LogP} + 0.013 \cdot A$  shown by the correlation between actual and predicted compound  $K_f$  values for (A) the experimental values used to build the model and (B) the independent replication values.

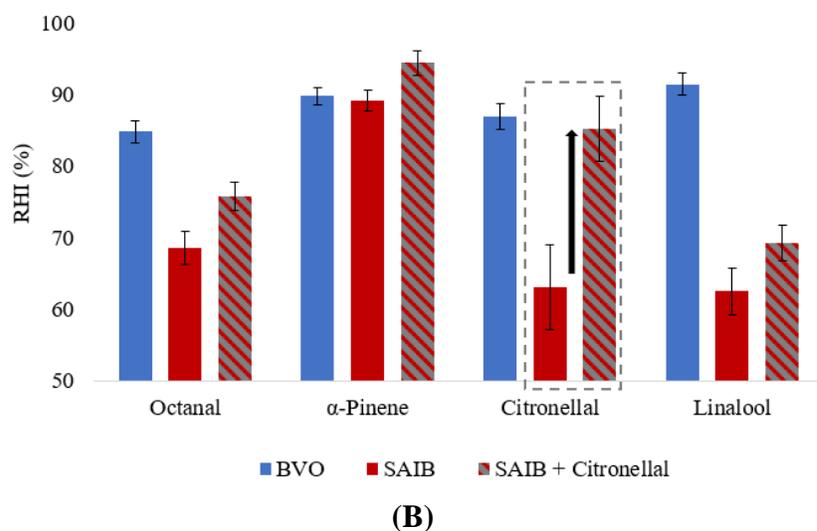
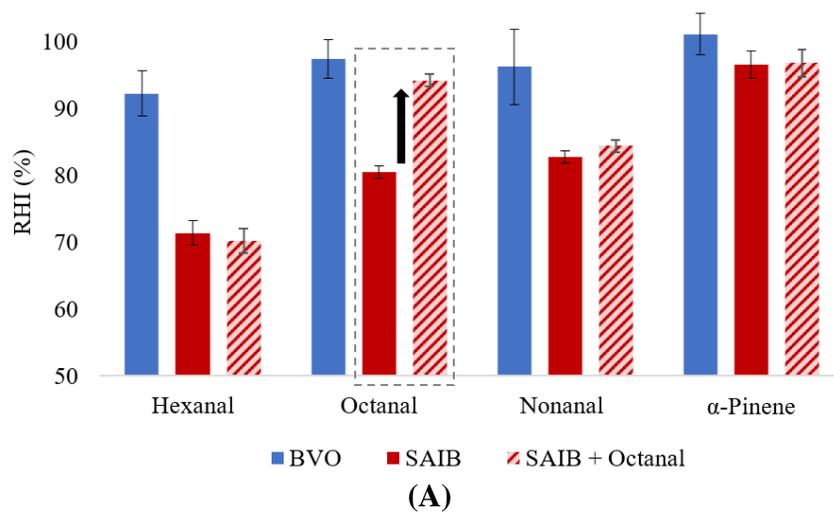


Figure 3.6. Volatility profiles and impacts of spiking within orange oil blends with BVO (blue) and SAIB (red) at usage ratios with comparison to SAIB A) spiked with 100ppm octanal (striped) and b) spiking with 25 ppm citronellal (striped). Error bars represent one standard deviation of each unit, which were run in triplicate.

Table 3.1. Weighting agent properties, and qualities within orange oil blends of typical usage ratios (the Newtonian viscosity ( $\mu$ , mPa\*s); kinematic viscosity ( $\nu$ , m<sup>2</sup>/s); density of mixture ( $\rho$ , g/mL)).

Abbreviation	Name	$\rho$ (g/mL)	Within Orange Oil Blend		
			$\mu$ (mPa*s)	$\nu$ (m <sup>2</sup> /s)	$\rho$ (g/mL)
SAIB	Sucrose Acetate	1.14	6.5	0.0069	0.9340
	Isobutyrate				
BVO	Brominated Vegetable Oil	1.33	2.5	0.0028	0.8802
EG	Glycerol Ester of Wood	1.08	13.4	0.0144	0.9256
	Rosin				

Table 3.2. Orange oil volatile compounds measured for each of the WAs at usage ratios with a comparison to the molecular properties logP and A (molar refractivity).

Compound	Classification	RHI with WA (%)			LogP	A (cm <sup>3</sup> )
		SAIB	BVO	EG		
$\alpha$ -Pinene	Terpene	93	96	93	4.37	44
$\beta$ -Pinene	Terpene	88	95	93	4.37	43.8
$\beta$ -Myrcene	Terpene	90	96	98	4.58	47.6
2-Carene	Terpene	93	94	110	4.37	44
3-Carene	Terpene	94	95	96	4.27	44
4-Carene	Terpene	91	98	105	4.37	44
Camphene	Terpene	83	86	91	4.37	43.8
$\beta$ -Ocimene	Terpene	82	90	98	4.70	47.9
$\gamma$ -Terpinene	Terpene	101	106	113	4.36	45.4
Linalool	Alcohol	66	91	86	3.28	49.5
$\alpha$ -Terpineol	Alcohol	64	81	77	2.79	47.1
Hexanal	Aldehyde	67	89	80	1.97	30
Octanal	Aldehyde	78	95	73	3.03	39.3
Nonanal	Aldehyde	77	92	76	3.56	43.9
Decanal	Aldehyde	82	86	59	4.09	48.6
Citronellal	Terpenoid	47	81	76	3.48	48.4
Citral (Neral)	Terpenoid	42	70	86	3.17	48.4
Citral (Geranial)	Terpenoid	49	65	91	3.17	48.4

Table 3.3. Clusters RHI for each of the WAs at usage ratios in orange oil with a comparison of the molecular properties.

<b>Cluster</b>	<b>RHI with WA (%)</b>			<b>Relative Change*</b>	<b>LogP</b>	<b>A (cm<sup>3</sup>)</b>
	<b>SAIB</b>	<b>BVO</b>	<b>EG</b>			
Terpenes	91	95	99	↓	4.42	44.99
Saturated Aldehydes	76	91	72	↓↓	3.03	39.30
Alcohols	65	86	81	↓↓	3.02	47.97
Terpenoids	46	72	84	↓↓↓	2.99	47.40

\*The number and direction of arrows reflects the relative magnitude and direction of change

Table 3.4. List of Abbreviations

<b>Abbreviation</b>	<b>Definition</b>	<b>Units</b>
BVO	Brominated Vegetable Oil	
K <sub>f</sub>	Complex Formation Constant	dimensionless
EG	Ester Gum	
HS	Headspace	
A	Molar Refractivity	cm <sup>3</sup>
logP	Octanal-water partition coefficient	dimensionless
RHI	Relative Headspace Intensity	%
SAIB	Sucrose Acetate Isobutyrate	
WA	Weighting Agent	

## CHAPTER 4: MOLECULAR INFLUENCES OF WEIGHTING AGENTS AND THE IMPLICATIONS ON COMPLEXATION WITH ORANGE OIL AROMA COMPOUNDS

Kelsey Kanyuck, Chris Pernell, and Christopher Daubert

[to be submitted to *Journal of Agricultural and Food Chemistry*]

### 4.1. Abstract

Beverage emulsions often require the addition of weighting agents (WAs) to flavor oils to prevent phase separation. WAs increase the density and amount of oil phase, inhibiting gravitational separation of the emulsion. Consumer complaints have called for the removal of the WA brominated vegetable oil (BVO) from beverages, and has motivated beverage companies to evaluate changes in flavor oil by switching to other WAs such as sucrose acetate isobutyrate (SAIB). This study utilized chemical analogs to compare molecular structures of BVO and SAIB and their effects on flavor compounds within orange oil. Specifically, the backbone, chain length, and brominated properties were compared between analogs. Each analog, at 0.52 m and 1.0 m, was dissolved in essential orange oil, and volatile constituents of the flavor oil blend were measured by headspace gas chromatograph mass spectrometry (GC-MS). Volatile compounds were normalized to compare relative headspace intensity (RHI), and changes between analogs were assessed. Large changes were detected in flavor compounds between the backbone analogs and the bromination analogs, but only minor between the chain length analogs. Different flavor compounds displayed different strength in associations, and these differences appeared to be dependent on chemical structure. Similarities in flavor compound changes between SAIB and BVO, but large differences in chemical structure, suggested dispersive interactions may be

responsible for the measured association. It was proposed that the number of excess polarizable (EP) bonds of a WA represent the binding sites and therefore the magnitude of effect of the WA.

## **4.2. Introduction**

Flavor oils used in beverage formulations often require the addition of weighting agents (WAs) to prevent separation. Due to the immiscibility of flavor oils in the aqueous-based beverage, the flavor is dispersed in the system by forming an emulsion (Tan, 2004). An emulsifier (often gum acacia) and homogenization help to stabilize the emulsion, but the addition of a WA is needed to sufficiently inhibit gravitational separation of the oil and aqueous phases (Chanamai and McClements, 2000). To this accord, Stokes Law (Eq. 2.1) demonstrates the impacts that WAs (which minimizes the difference in density) and emulsifier with homogenization (which minimizes the particle size) have on gravitational separation (McClements, 2016). Several studies have previously confirmed the effectiveness of WAs on decreasing the rate of emulsion creaming (Tse and Reineccius, 1995; Trubiano 1995; Chanamai and McClements, 2000; Lim et al. 2011).

Flavor oils for orange-flavored and other citrus beverages typically contain essential oils. Essential orange oil is extracted from the peel of the orange citrus fruit and contains numerous volatile compounds that produce the characteristic orange flavor. The composition of orange oil consists mainly of terpene molecules, such as limonene, and the other classes of flavor compounds are present in the oil at low ratios (< 2% w/w) such as alcohols, aldehydes, esters, and terpenes (Shaw, 1979; Hognadottir and Rouseff, 2003; Qiao et al. 2008; Perez-Cacho and Rouseff, 2008). Small differences in composition can contribute to perceived differences in flavor, as evidenced by the similarity in composition of flavors molecules

between various citrus varieties (lime, lemon, orange), but uniqueness in flavor perception (Shaw, 1979; Perez-Cacho and Rouseff, 2008). The volatile compounds utilized in this experiment are shown and explained in further detail in Table 2.3, 2.4, and 4.2. Essential oils have a density around 0.86 g/mL (Chapter 3) and thus require the addition of WAs to reach an oil phase density closer to that of the aqueous phase.

Two common WAs are sucrose acetate isobutyrate (SAIB) and brominated vegetable oil (BVO). The ban of BVO in Europe (Tan, 2004) has motivated manufacturers to switch to alternative WAs. An alternative WA, SAIB is a mixture of esterified sucrose molecules with an average esterification of two acetate and six isobutyrate moieties per sucrose (pictured in Figure 4.1). BVO is a triglyceride, with an average chain length of 18 carbons that has been saturated with 2-4 bromine atoms on each fatty acid (pictured in Figure 4.1) (Bendig et al. 2012). Switching WAs in a flavor oil has been demonstrated to modify headspace concentrations of volatile components (Chapter 3), but the chemical basis of these associations has not been addressed. The switch from a lipid-derived, brominated molecule (BVO) to a carbohydrate-derived octa-acetylated molecule (SAIB) presents a change in polarity, polarizability, spatial orientation, molecular moieties, and density. The chemistry of these two WAs were compared using pair-wised comparisons of several sets of analogs to elucidate the structural basis of associations with flavor compounds. Understanding the function of structural components of WAs will assist manufacturers in modifying or creating new WAs for replacement of BVO, without changing the flavor profile.

Past research on changing the type or concentration of lipids in food products has demonstrated changes in partitioning of flavor molecules, and the extent of which was often dependent on the chemistry of flavor compounds. The increase in a hydrophobic additive has

been shown to decrease flavor oil (Malone et al. 2000; Philippe et al. 2003; Arancibia et al. 2011) and essential oil (Chang et al. 2012) component partition out of the oil phase. Using trained panel sensory analysis, a 30% oil phase emulsion was found to have a lower citrus flavor than the 5% oil phase, correlating with the linalool concentration (Arancibia et al. 2011). More relevant, a WA (ester gum) was suggested to decrease the solubility of oil phase compounds into the aqueous phase of an emulsion by slowing Ostwald Ripening (McClements et al. 2012). Decreased availability of oil components, based on higher additive concentration, has been attributed to greater oil partitioning and an increase in entropy of the oil phase due to mixing (McClements, 2016; Chang et al. 2012).

In addition to concentration, the type of additive and chemical properties have been suggested to impact the partitioning of oil components (Fabre et al. 2006; Philippe et al. 2003; Chang et al. 2012; Mao et al. 2014). At the same concentration (by wt) in emulsions, medium chain triglyceride (MCT) oil decreased the release of oil components more than that of soybean oil (with longer chain lengths), suggesting different partition coefficients (Mao et al. 2014). Similar effects were observed by Chang et al. (2012) with corn oil and MCT, and researchers have hypothesized the difference is due to variances in lipophilicity, mole fractions, or fatty acid saturation levels (Chang et al. 2012; Mao et al. 2014). The effect on individual flavor compounds is consistently reported as dependent on the hydrophobicity (logP) and chemical properties (Philippe et al. 2003; Relkin et al. 2004; Mao et al. 2014; Arancibia et al. 2011). Philippe et al. (2003) concluded that physicochemical interactions occurred between the flavor compounds and oil phase, causing differences in partitioning. Within flavor oil blends, Chapter 3 suggested the associations between WAs and flavor compounds were controlled by the polarity and polarizability of flavor compounds, as well as

the concentration of WA. Due to the nature of emulsions, the partitioning coefficients of the oil components are primarily based on the water solubility, oil phase interactions, and compound volatility. In the present experiment, water was not part of the system, and thus changes in volatility were only caused by oil phase interactions and compound volatility (the last of which is constant at a maintained temperature, for a given compound).

The objective of this chapter was to assess chemical differences between SAIB and BVO by comparing chemical analogs to attribute the contributions of molecular differences on flavor compound complexations. An experimental design was utilized to determine which parts of the molecular chemistry may be responsible for the differences in flavor compound associations between SAIB and BVO (as demonstrated in Chapter 3). Differences in molecular structure were simplified down to three variables: backbone (sucrose vs. glycerol), chain length ( $C_4$  vs  $C_8$  vs  $C_{18}$ ), and bromination (presence vs. absence). The experimental design, with each pairwise comparison, is shown graphically in Table 4.1. The ‘backbone’ variable includes several structural differences (number of chains attached, orientation, size, and number of ester bonds), and the impacts of which were studied together, as one variable, for simplicity and feasibility. Various compounds with differing chemical structures were dissolved in orange oil to elucidate the impact of the chemical structures of each analog. These analogs were not proposed as replacement WAs (they have low density), but instead analogs were chosen because they each contain molecular properties that allowed direct comparison of the three parameters of interest (backbone, chain length, and bromination). This chapter will address the implications of WA chemistry on flavor compound interactions. Knowledge of the influential chemical structures in associations with flavor compounds will

be important for formulating new WAs that can replace BVO without modifying the volatility of flavor compounds.

### **4.3. Materials and Methods**

#### **4.3.1 Flavor Oil Blends**

Mixtures of orange oil and WA analogs were prepared to compare direct effects of each additive on flavor compound volatility using analogs shown in Table 4.1. Due to availability and miscibility, three analogs were blends of several components, but all calculations assumed pure mixtures of the primary component. For instance, SAIB is not purely a sucrose octa-butyrate, but instead a mixture of sucrose esters with isobutyrate and acetate moieties at varying degrees of esterification. Compounds used to approximate each WA analog were glyceryl tributyrate (GlyC<sub>4</sub>), glyceryl trioctanoate (GlyC<sub>8</sub>), corn oil (GlyC<sub>18</sub>), SAIB (SucC<sub>4</sub>), and BVO (GlyC<sub>18</sub>Br<sub>2</sub>). Glyceryl tributyrate and glyceryl trioctanoate with  $\geq 99\%$  purity were purchased from Sigma-Aldrich (St. Louis, MO). The BVO was purchased from Sigma Chemical Manufacturing Corp. (New Brunswick, NJ), and the SAIB was a donation from Eastman Chemical Company (Kingsport, TN). Corn oil was purchased from a local supermarket. The orange flavor oil was 5x essential orange oil purchased from Berjé Inc. (Carteret, NJ). WA analogs were dissolved in orange oil by adding each compound to orange oil in a capped glass vial. Concentrations of 1.0 molality (m) and 0.52 m were prepared for each of the analogs, and orange oil was considered the control. Once the flavor oil blends were formulated, the blends were equilibrated at 25°C for 96 hours while shaking at 1.0 rpm and later analyzed for relative ratios of key compounds in the headspace. Blends were created in triplicate and each sample run in duplicate, for a total sample size of six per variable.

#### 4.3.2 Volatility Measurements

Headspace gas chromatography - mass spectrometry (HS GC-MS) was used to measure the volatility of flavor compounds in the orange oil blends. A 7820A GC coupled with a 5977E quadrupole MSD (Agilent Technologies, Santa Clara, USA) was used to separate and quantify the volatile components of the samples. The sampling method utilized a CTC CombiPAL autosampler (Lake Elmo, MN) to withdraw 1.0 mL of HS from 20.0 mL glass vials with 50.0  $\mu$ L of oil blend which had been equilibrated at 50.0°C for 20 minutes. The withdrawn headspace was immediately injected into the GC inlet with at 1:10 split at 275 °C and onto a J&W HP-5 MS column (30 m $\times$ 0.25 mm I.D, 0.25  $\mu$ m film thickness (Agilent Technologies, Santa Clara, USA)). With helium gas at a constant flow rate of 1.2 mL/min, the temperature ramp of the GC began with a 3 minute hold at 40 °C and was set to increase at 3°C/min until 115°C with a 1 minute hold at 71°C and then increased at 45°C/min until 250°C with a 1 minute hold. Mass spectra were obtained in TIC mode and counted ions from 30–250 m/z in increments of 0.1 m/z. Using the MS fragmentation and retention index (RI) of each peak reported by Adams (1989) and Qiao et al. (2008), compounds were tentatively identified. Chemical standards confirmed the identification of limonene,  $\beta$ -pinene, linalool, hexanal, and octanal (Sigma Aldrich, St. Louis, MO). The list of molecules detected, identified (tentatively), and used for analysis is displayed in Table 4.2.

Modifications in volatility for each flavor compound were detected by alterations in the HS concentration and measured by changes in peak area. For each molecule, peak areas were converted to relative headspace intensity (RHI) to normalize for the wide range of volatile concentration naturally present in the flavor oils (Eq. 3.1). Additionally, a complex formation constant ( $K_f$ ) was calculated for each flavor compound and analog pair as

described in Eq 2.5. Flavor compounds detected in the orange oil were categorized into several chemical classes in accordance with the clusters identified in Chapter 3. These analyses allowed for simplification and comparison between flavor compounds and analogs.

#### **4.4. Results and Discussion**

Volatile concentrations of key flavor compounds were analyzed in each orange oil blend to measure changes in volatility of individual molecules. Compounds were grouped into clusters by chemical class, allowing for simplified analysis and visualization (as identified in Chapter 3). Due to large amounts of volatile material in these samples, the HS concentration was a measure of partial pressures and not a true measurement of concentration. Additionally, in each oil blend, flavor compounds were competing for ‘binding sites’ or interactions with WA analogs, and thus individual flavor compound changes were not truly independent. The WAs are commonly added to flavor oils at these ratios, and as such, the analyzed influences of these agents on the flavor blend properties are representative of the non-ideal system used in beverages.

##### **4.4.1. Importance of Additive Concentration**

Each chemical analog was analyzed at concentrations of 0.52 m and 1.0 m in orange oil, and the results are summarized in Table 4.2. There was a decrease in volatility with increasing WA analog for linalool and  $\beta$ -pinene, with a greater decrease in the RHI of linalool (Figure 4.2). For each analog, the RHI of the terpenoid and alcohol flavor compounds were significantly lower at the 1.0 m than the 0.52 m concentration (Table 4.2), indicating the importance of concentration. Regardless of chemical structure, a trend of decreasing RHI with increasing additive concentration was observed in this and prior work (Chapter 3). Other experiments have previously observed decreasing solubility or volatility

of flavor oil compounds with the addition of a non-polar additive, and the type of additive often makes a difference (Harrison et al. 1997; Carey et al. 2002; van Ruth et al. 2002; Malone et al. 2000; Leksrisompong et al. 2010). For instance, Chang et al. (2012) demonstrated that different compounds added to the oil phase (both the type and concentration) had different impacts of the extent of antimicrobial activity of an essential oil. Furthermore, these authors suggested the cause was a greater partitioning of the active agent when a larger amount of added oil was present. Chapter 3 found a comparable result that both type and concentration of WA impacted flavor volatility. Because the usage level of WAs is different, it is reasonable to assume that agents added in application at differing concentrations would produce differences in volatile profiles.

#### 4.4.2. Comparison of Weighting Agents

Figure 4.3A shows the comparison of flavor compound interactions between inclusion of SAIB and BVO. Each dot represents a single flavor compound, the line of equality shows where the  $K_f$  is the same between SAIB and BVO, and the star represents no change from the control (at 100% RHI). Generally, the flavor compounds appeared to follow the line of equality, suggesting similar effects of SAIB and BVO at normalized molality. Clusters identified in Chapter 3 were applied to Figure 4.3A and are shown by colored circle overlays in Figure 4.3B. In this graph, termed a cluster plot, the center of each circle signifies the centroid of the cluster, and the circle area reflects the relative standard deviation. Terpene class molecules displayed the lowest association to both WA analogs, as evidenced by the smallest  $K_f$  values. Conversely, the terpenoid class compounds had the highest association (Figure 4.3 and Table 4.3) which was consistent with previous analysis (Chapter 3). The

consistency in ordering of  $K_f$  by class suggested that similar interactions caused the associations with both SAIB and BVO.

If any compounds appeared to significantly deviate from the line of equality, it would have suggested a stronger interaction with that particular WA. Because BVO and SAIB interacted similarly with most compound classes at normalized concentrations, a variation is likely at dissimilar concentrations. Due to differences in density of the WAs, the typical concentration in an oil blend of SAIB is 0.52 m and BVO is 0.04 m (Chapter 3). Based on the effects of additive concentration (Figure 4.2) the disproportion in usage level would cause different binding profiles. In fact, Chapter 3 found stronger binding to SAIB than BVO at typical usage levels, as these trends predicted. Differences between SAIB and BVO will be further explored through comparison of the differing molecular structures.

#### 4.4.3. Influences of Flavor Molecule Chemistry

Volatile compounds were not impacted equally by the WA analogs, but instead it appeared that the most associative molecules were consistently the most affected for all analogs (Table 4.2). Variations between flavor compound associations necessitated an analysis approach that considered the flavor compound differences as well as the WA analog differences. Addressing the implications of WA analogs with a multivariate approach, the different flavor compounds were grouped by previously defined clusters and averages displayed in Table 4.3. The analogs showed preferential association to molecules with an oxygen atom, suggesting this atom is a key part of the association. Terpenoid molecular structure consists of a single aldehyde group and two carbon double bonds on a branched carbon chain, while the alcohols consist of an alcohol group instead of the aldehyde group, and the linear aldehydes do not contain any carbon double bonds. The terpene, alcohol, and

terpenoid compounds used in analysis all consisted of ten carbons with different orientations and some with oxygenated groups. Therefore, the major differences in structure (orientation or oxygenated group) appeared to cause the observed differences in association between the WA analogs. Favorability of associations with terpenoids over alcohols or saturated aldehydes may allow further insight into the molecular orientation of these interactions.

Molecular interactions between additives and individual flavor compounds have been previously studied. In an oil system, Genovese et al. (2015) measured a decrease in volatility of flavor compounds with the phenolic compound addition to virgin olive oil and hypothesized the associations were caused by complexation, because of the dependence of flavor compound chemistry. For linalool complexation with cyclodextrin, the site of interaction was determined to be the alcohol group, using X-ray crystallography (Ceborska, 2016). Additionally, Ceborska (2016) found that linalool and other linear alcohols could pack more tightly than ring-structured alcohols due to the better fit of the shape within the cyclodextrin binding pocket. Other studies corroborated the effects of cyclodextrin binding pocket size on the association of flavor compounds (Reineccius et al. 2005, Astray et al. 2010). Although the structures of cyclodextrins are not comparable to WA analogs, the active binding site of linalool is likely to be the same in both, and these types of interactions may provide insight into WA analog associations. Regarding WA associations specifically, Chapter 3 modeled the association between flavor compounds and SAIB which found that polarity and polarizability were likely driving factors of the interaction. Based on these past studies, the binding or association of flavor molecules to an additive have been shown to occur through molecular interactions by specific functional groups on both compounds.

The consistency of cluster ordering and linearity suggests associations were caused by the same type of interactions with the analogs, with differences only in magnitude. Dispersion associations are a type of induced-dipole molecular interaction that each of these flavor compounds would be expected to participate in. Dispersion forces are typically the largest contribution to attractive interactions, except when molecules are very polar, which was not the case in this system (Israelachvili, 2011). Molecular polarizability, a measure of the ease of displacement of electrons within a bond or molecule, can be used to account for differences in dispersion forces (Israelachvili, 2011). Therefore, the degree of the polarizability of these bonds may account for the differences between the chemical classes. An aldehyde group and two double bonds (on a terpenoid molecule) has the highest total polarizability, while the two double bonds on most terpenes with no oxygen bond have the lowest measure of polarizability (Israelachvili, 2011). Dispersive associations with WA analogs could explain the difference between flavor compounds, and may also be able to account for differences between WA analogs.

#### 4.4.4. Molecular Structure Importance Through Analog Comparison

On average, all flavor compounds interacted more strongly with SAIB or BVO than any of the glycerol backbone compounds, which may suggest that backbone and the bromines play a role in the interactions (Table 4.3). Considering the  $K_f$  value normalizes for the concentration of weighting agent, these graphs represent a per-mole comparison not necessarily an equal volume or equal mass comparison. Further analog comparisons will elucidate the key structural differences between these two WAs by 1) backbone, 2) chain length, and 3) bromination.

*Effects of Compound Backbone:* The effects of WA analog backbone (sucrose and glycerol) are shown in Figure 4.4 with the pairwise comparison of SucC<sub>4</sub> (SAIB) and GlyC<sub>4</sub>. Although the cluster plot showed the same general cluster order as Figure 4.3, the clusters deviated from the line of equality. Stronger association to SucC<sub>4</sub> (SAIB) than GlyC<sub>4</sub> suggested the sucrose backbone produced greater binding to flavor compounds, specifically the oxygenated molecules, which were consistently most impacted. The backbone variable included several parameters such as size, number of ester bonds, number of chains, orientation, and the steric hindrance of a binding pocket. The influences of specific parameters within the backbone cannot be looked at independently in this comparison, but instead are considered as one variable. Likely causes of the influence of molecular backbone may include a greater number of binding sites (more ester groups per molecule) or steric arrangement of chains which impacted interactions with the backbone.

*Effects of Compound Chain Length:* Implications of side group chain length (4 carbon, 8 carbon, and 18 carbon) are shown with the plot of GlyC<sub>4</sub> vs. GlyC<sub>18</sub> in Figure 4.5, while results from all three glycerol are displayed in Table 4.2. Lack of separation between compounds and alignment along the equality line indicates minimal differences between analogs and a relatively low overall impact on flavor volatility (in comparison to the other two variables compared). The similarity of the four clusters emphasizes the glycerol backbone analogs did not appear to produce strong differentiation between compounds (Figure 4.5), suggesting either the preferences were clouded by the lack of interaction, or rather these analogs do not favor association with certain classes of flavor compounds. This study is not able to support either hypothesis, but it may be of future interest.

From this experimental observation, it appears that chain length does not impact flavor compound interactions, and this further emphasized that associations take place at polarizable regions of the WA. Binding of the three glycerol backbone analogs was lower than SucC<sub>4</sub> associations (Table 4.3). The three glycerol analogs all had similar effects, suggesting that the ‘chain length’ variable is not a main contributor to the flavor compound interactions. There may have been a slight preference for the GlyC<sub>18</sub> analog, caused by fatty acids in the mixture that do contain double bonds (which have excess polarizability), but it does not appear to be substantial. It should not be assumed there is no interaction between backbone and chain length variables, and sucrose with longer chains must be separately analyzed to make a full conclusion. Based on the three chain lengths of glycerol backbone, additional chain length only added alkane bonds which have low polarizability. The large increase in low-polarizability bonds showed minimal difference in flavor compound binding, further suggesting that associations with flavor compounds occur at polarizable regions.

*Effects of Compound Bromination:* Effects of bromination on flavor compound interactions with WA analogs is displayed in Figure 4.6 with a comparison of GlyC<sub>18</sub>Br<sub>2</sub> to GlyC<sub>18</sub>. In agreement with the other cluster plots, terpenoids had the highest association and terpenes had the lowest association to both analogs. The deviation from the line of equality towards GlyC<sub>18</sub>Br<sub>2</sub> (BVO) suggested bromination increases interactions of the oxygenated flavor molecules. Bromine molecules are known to be highly polarizable (Thomas et al. 2015), suggesting the association with flavor compounds may be driven by polarizability. The bromination of vegetable oil increased association with flavor compounds, and was suggested to be caused by a greater molecular polarizability.

*Overall Effects:* From conclusions of each chemical analog comparison, the backbone and bromination parameters were likely contributing to associations with flavor compounds. The sucrose backbone and the presence of bromination increased binding with flavor compounds (higher  $K_f$ ), while chain length was not suggested to be an important influence. Both the sucrose backbone and bromination parameters add polarizable bonds to the WA analog. An excess polarizability (EP) measurement can be used to compare the polarizability of analogs in relation to the bulk or solvent polarizability (Israelachvili, 2011). In flavor oil and WA blends, a carbon-carbon single bond is most prevalent. Thus, for analogs with bond polarizabilities greater than an alkane (0.48), the difference was summed together as a measure of the bond EP. An ester bond had an EP of 1.98, an ether bond 0.62, a bromine-carbon bond 2.93, and a carbon-carbon double bond 1.17 with units  $(4\pi\epsilon_0)10^{-30} \text{ m}^3$  (Israelachvili, 2011). The summation of EP (see Table 4.2) did not appear to correlate with higher association with WA, but the number of bonds with EP appeared to be associated with  $K_f$ . Analogues with sucrose backbone and bromination both had higher numbers of EP bonds than the directly compared analogs (11 compared to 3 respectively), and the same as each other (Table 4.4). In response, it was hypothesized that the number of EP bonds was representative of the number of binding spots for each analog. A higher number of EP bonds would be able to accept more dispersion interactions with the polarizable groups of flavor compounds, and thus a greater measured impact. Accordingly, SucC<sub>4</sub> (SAIB) and GlyC<sub>18</sub>Br<sub>2</sub> (BVO) molecules displayed similar magnitudes of interactions due to an equivalent number of binding spots.

Different usage molalities would alter the number of EP bonds in solution and thus the extent of binding with flavor compounds. In one mole, the number of EP bonds is

dependent on the size and type of molecule. Changing the number of molecules (such as different concentrations) would also alter the total EP of the WA analog. For instance, four times the number of molecules of GlyC<sub>4</sub> would have a similar number of EP bonds to GlyC<sub>18</sub>Br<sub>2</sub> (BVO). This proposed mechanism would explain the similarity between SucC<sub>4</sub> (SAIB) and GlyC<sub>18</sub>Br<sub>2</sub> (BVO) at the same molality, despite their structural differences. At the same molality, SucC<sub>4</sub> (SAIB) and GlyC<sub>18</sub>Br<sub>2</sub> (BVO) would affect flavor volatility similarly, due to the similar number of bonds with excess polarizability, but different concentrations (typical usage ratios) create different number of EP bonds. To match the associative effects of BVO, a new WA should have equal number of polarizable groups at the usage concentration. For instance, SAIB could be used at equal molality to match the EP bonds. Knowledge of the number of EP bonds would allow for vastly different usage ratios between WAs, because if the total number of EP bonds is maintained, interactions with a flavor oil are expected to display similar amounts of interactions.

Other parameters of these analogs, such as logP, the polar surface area (PSA), or molar refractivity (A) were not able to account for the polarizability of the Br-C bond, but instead used the polarity of the bond (which is considered quite low). Thus, the ACD estimated values in Table 4.4 do not accurately describe the interactions of GlyC<sub>18</sub>Br<sub>2</sub> (BVO) because polarizability was not considered. Instead, the number of EP bonds does account for the polarizability of the Br-C bonds and appears to correlate with the K<sub>f</sub> for flavor compounds. The proposed mechanism of number of EP as binding spots does not account for differences in orientation or bulkiness, which may also impact binding affinities. Generally, the number of polarizable groups on a WA were suggested to be a driving factor in K<sub>f</sub> with flavor compounds.

## 4.5. Conclusion

The backbone and bromination of the WAs were suggested to contribute to differences in flavor compound complexation, while the chain length was not. Both the concentration and the chemistry of the WA affected the interactions with flavor compounds, and the chemistry of flavor compounds also played a role. At normalized molality, the extent of associations between SAIB (SucC<sub>4</sub>) and BVO (GlyC<sub>18</sub>Br<sub>2</sub>) were similar (for terpenoids, aldehydes, alcohols, and terpenes) but at usage ratios there is an expected greater association to SAIB than BVO. It was proposed that the number of EP of bonds can account for differences between WA analog interactions. Both variables that increased the number of EP bonds (backbone and brominated) resulted in greater associations to flavor compounds, and higher concentration also increased the number of EP bonds. The chain length does not modify the number of EP bonds, and was consequentially not suggested to be an important structural attribute. Although differences in compound chemistry and concentration were suggested to be significant, the implications on flavor perception has yet to be evaluated. Beverage manufacturers that wish to include a new WA are suggested to maintain the number of EP bonds of the current WA to minimize changes in flavor compound volatility.

#### 4.6. References

- Adams RP. 1995. Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, Ill. : Allured Pub. Corp.
- Arancibia C, Jublot L, Costell E, Bayarri S. 2011. Flavor release and sensory characteristics of o/w emulsions. Influence of composition, microstructure and rheological behavior. *Food Res.Int.* 44(6):1632-41.
- Astray G, Mejuto JC, Morales J, Rial-Otero R, Simal-Gándara J. 2010. Factors controlling flavors binding constants to cyclodextrins and their applications in foods. *Food Res.Int.* 43(4):1212-1218.
- Bendig P, Maier L, Vetter W. 2012. Brominated vegetable oil in soft drinks - an underrated source of human organobromine intake. *Food Chem.* 133(3):678-682.
- Carey ME, Asquith T, Linforth RS, Taylor AJ. 2002. Modeling the partition of volatile aroma compounds from a cloud emulsion. *J.Agric.Food Chem.* 50(7):1985-90.
- Ceborska M. 2016. Structural investigation of the  $\beta$ -cyclodextrin complexes with linalool and isopinocampheol – Influence of monoterpenes cyclicity on the host–guest stoichiometry. *Chemical Physics Letters.* 651:192-197.
- Chanamai R, McClements DJ. 2000. Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J.Agric.Food Chem.* 48(11):5561-5.
- Chang Y, McLandsborough L, McClements DJ. 2012. Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening inhibitors. *J.Agric.Food Chem.* 60(48):12056-63.
- Fabre M, Relkin P, Guichard E. 2006. Flavor Release from Food Emulsions Containing Different Fats. *ACS Symp.Ser.* (920):61-72.
- Genovese A, Caporaso N, Villani V, Paduano A, Sacchi R. 2015. Olive oil phenolic compounds affect the release of aroma compounds. *Food Chem.* 181:284-294.
- Harrison M, Hills BP, Bakker J, Clothier T. 1997. Mathematical Models of Flavor Release from Liquid Emulsions. *Journal of Food Science -Chicago-* 62(4):653-658.
- Högnadóttir A, Rouseff RL. 2003. Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *Journal of chromatography.A* 998(1-2):201-11.

- Israelachvili JN. 2011. *Intermolecular and Surface Forces: Revised Third Edition* (3). US: Academic Press, 2011. ProQuest ebrary. Web. 8 November 2016.
- Leksrisompong PP, Barbano DM, Foegeding AE, Gerard P, Drake MA. 2010. The Roles Of Fat And Ph On The Detection Thresholds And Partition Coefficients Of Three Compounds: Diacetyl,  $\Delta$ -Decalactone And Furanol. *J.Sens.Stud.* 25(3):347-370.
- Lim SS, Baik MY, Decker EA, Henson L, Michael Popplewell L, McClements DJ, Choi SJ. 2011. Stabilization of orange oil-in-water emulsions: A new role for ester gum as an Ostwald ripening inhibitor. *Food Chem.* 128(4):1023-1028.
- Malone ME, Appelqvist IAM, Goff TC, Homan JE, Wilkins JPG. 2000. A Novel Approach to the Selective Control of Lipophilic Flavor Release in Low Fat Foods. *ACS Symp.Ser.* 763212-229.
- Mao L, Roos YH, Miao S. 2014. Flavour Release from Monoglyceride Structured Oil-in-Water Emulsions through Static Headspace Analysis. *Food Biophysics* 9(4):359-367.
- McClements DJ, Henson L, Popplewell LM, Decker EA, Jun Choi S. 2012. Inhibition of Ostwald Ripening in Model Beverage Emulsions by Addition of Poorly Water Soluble Triglyceride Oils. *J.Food Sci.* 77(1):C33-C38.
- McClements DJ. 2016. *Food Emulsions : principles, practice, and techniques*. 3rd ed. Boca Raton : CRC Press, Taylor & Francis Group.
- Philippe E, Seuvre AM, Colas B, Langendorff V, Schippa C, Voilley A. 2003. Behavior of flavor compounds in model food systems: a thermodynamic study. *J.Agric.Food Chem.* 51(5):1393-8.
- Qiao Y, Xie BJ, Zhang Y, Fan G, Yao XL, Pan SY. 2008. Characterization of aroma active compounds in fruit juice and peel oil of Jincheng sweet orange fruit (*Citrus sinensis* (L.) Osbeck) by GC-MS and GC-O. *Molecules* (Basel, Switzerland) 13(6):1333-44.
- Perez-Cacho PR, Rouseff RL. 2008. Fresh squeezed orange juice odor: a review. *Crit.Rev.Food Sci.Nutr.* 48(7):681-95.
- Reineccius TA, Reineccius GA, Peppard TL. 2005. The effect of solvent interactions on alpha-, beta-, and gamma-cyclodextrin/flavor molecular inclusion complexes. *J.Agric.Food Chem.* 53(2):388-92.
- Relkin P, Fabre M, Guichard E. 2004. Effect of fat nature and aroma compound hydrophobicity on flavor release from complex food emulsions. *J.Agric.Food Chem.* 52(20):6257-63.

Shaw PE. 1979. Review of quantitative analyses of citrus essential oils. *J.Agric.Food Chem.* 27(2):246-257.

Tan, C. 2004. Beverage Emulsions. In: Larsson, K., & Friberg, S. *Food emulsions*. 4th ed., rev. and expanded. New York: M. Dekker. 485-524.

Thomas MJ, Bramblett KA, Green BD, West KN. 2015. Thermophysical and absorption properties of brominated vegetable oil. *Journal of Molecular Liquids* 211647-655.

Trubiano PC. 1995. The Role of Specialty Food Starches in Flavor Emulsions. *ACS Symp.Ser.* 610199-209.

Tse KY, Reineccius GA. 1995. Methods To Predict the Physical Stability of Flavor-Cloud Emulsion. *ACS Symp.Ser.* 610172-182.

van Ruth SM, King C, Giannouli P. 2002. Influence of lipid fraction, emulsifier fraction, and mean particle diameter of oil-in-water emulsions on the release of 20 aroma compounds. *J.Agric.Food Chem.* 50(8):2365-71.

Table 4.1. Experimental design variables and ingredient comparisons used to contrast each variable.

<b>Ingredient Analog</b>	<b>Abbreviation</b>	<b>Backbone</b>	<b>Variable</b>	
			<b>Chain Length</b>	<b>Bromination</b>
Sucrose Acetate Isobutyrate (SAIB)	Suc C <sub>4</sub>	<b>Sucrose</b>	C <sub>4</sub>	No
Glyceryl tributyrate	Gly C <sub>4</sub>	<b>Glycerol</b>	<b>C<sub>4</sub></b>	No
Glyceryl trioctanoate	Gly C <sub>8</sub>	Glycerol	<b>C<sub>8</sub></b>	No
Corn Oil (Glycerol tristearate)	Gly C <sub>18</sub>	Glycerol	<b>C<sub>18</sub></b>	<b>No</b>
Brominated Vegetable Oil (BVO)	Gly C <sub>18</sub> Br <sub>2</sub>	Glycerol	C <sub>18</sub>	<b>Yes</b>

Table 4.2. Orange oil volatile compounds associations with chemical analogs as measured by  $K_f$  ( $m^{-1}$ ) for each individual compound.

<b>Compound</b>	<b>Classification</b>	<b>SucC<sub>4</sub></b>	<b>GlyC<sub>4</sub></b>	<b>GlyC<sub>8</sub></b>	<b>GlyC<sub>18</sub></b>	<b>GlyC<sub>18</sub>Br<sub>2</sub></b>
$\alpha$ -Pinene	Terpene	0.14	0.09	0.15	0.25	0.20
$\beta$ -Pinene	Terpene	0.14	0.12	0.11	0.20	0.21
$\beta$ -Myrcene	Terpene	0.12	0.10	0.09	0.12	0.11
2-Carene	Terpene	0.05	0.07	0.11	0.10	0.11
3-Carene	Terpene	0.06	0.05	0.07	0.14	0.12
4-Carene	Terpene	0.12	0.08	0.17	0.25	0.25
Camphene	Terpene	0.20	0.18	0.27	0.37	0.37
$\beta$ -Ocimene	Terpene	0.17	0.11	0.17	0.23	0.26
$\gamma$ -Terpinene	Terpene	0.13	0.10	0.17	0.28	0.28
Linalool	Alcohol	0.47	0.26	0.29	0.27	0.32
$\alpha$ -Terpineol	Alcohol	0.49	0.17	0.32	0.39	0.51
Hexanal	Aldehyde	0.66	0.38	0.39	0.55	0.68
Octanal	Aldehyde	0.35	0.27	0.24	0.24	0.35
Nonanal	Aldehyde	0.29	0.15	0.17	0.11	0.29
Decanal	Aldehyde	0.11	0.00	0.03	0.01	0.18
Citronellal	Terpenoid	0.45	0.22	0.32	0.37	0.52
Citral (Neral)	Terpenoid	0.56	0.16	0.30	0.37	0.72
Citral (Geranial)	Terpenoid	0.47	0.11	0.18	0.26	0.64

Table 4.3. Comparison of  $K_f$  for each flavor compound cluster with each of the WA chemical analogs.

<b>Analog</b>	<b>Average <math>K_f</math></b>			
	<b>Saturated</b>			
	<b>Terpenes</b>	<b>Aldehydes</b>	<b>Alcohols</b>	<b>Terpenoids</b>
Suc C <sub>4</sub> (SAIB)	0.15	0.35	0.48	0.49
Gly C <sub>4</sub>	0.12	0.20	0.22	0.16
Gly C <sub>8</sub>	0.16	0.21	0.30	0.27
Gly C <sub>18</sub>	0.24	0.23	0.33	0.33
Gly C <sub>18</sub> Br <sub>2</sub> (BVO)	0.23	0.37	0.42	0.63

Table 4.4. Molecular characterization of assumed pure compound in each chemical analog with molecular properties: EP, excess polarizability (per molecule); sum of EP (per molecule) with units  $(4\pi\epsilon_0)10^{-30} \text{ m}^3$ , logP; PSA; Polar Surface Area ( $\text{\AA}^2$ ); A, molar refractivity( $\text{cm}^3$ ).

<b>Analog</b>	<b>Molar Mass (g/mol)</b>	<b>Density (g/mL)</b>	<b>Molecular Properties</b>				
			<b>Bonds with EP (#)</b>	<b>Sum of EP<sup>b</sup></b>	<b>LogP<sup>a</sup></b>	<b>PSA<sup>a</sup></b>	<b>A<sup>a</sup></b>
Suc C <sub>4</sub> (SAIB)	846.9	1.14	11	17.7	8.46	238	203
Gly C <sub>4</sub>	302.4	1.03	3	5.9	2.95	79	77
Gly C <sub>8</sub>	470.7	0.96	3	5.9	9.33	79	132
Gly C <sub>18</sub>	891.5	0.86	3	5.9	25.3	79	271
Gly C <sub>18</sub> Br <sub>2</sub> (BVO)	1550.7	1.33	11	29.4	26.0	79	342

Source: <sup>a</sup> ACD labs <sup>b</sup> Israelachvili, 2011

Table 4.5. List of Abbreviations

<b>Abbreviation</b>	<b>Definition</b>	<b>Units</b>
BVO	Brominated Vegetable Oil	
K <sub>f</sub>	Complex Formation Constant	dimensionless
EG	Ester Gum	
EP	Excess Polarizability	$(4\pi\epsilon_0)10^{-30} \text{ m}^3$
HS	Headspace	
A	Molar Refractivity	$\text{cm}^3$
logP	Octanal-water partition coefficient	dimensionless
RHI	Relative Headspace Intensity	%
SAIB	Sucrose Acetate Isobutyrate	
WA	Weighting Agent	

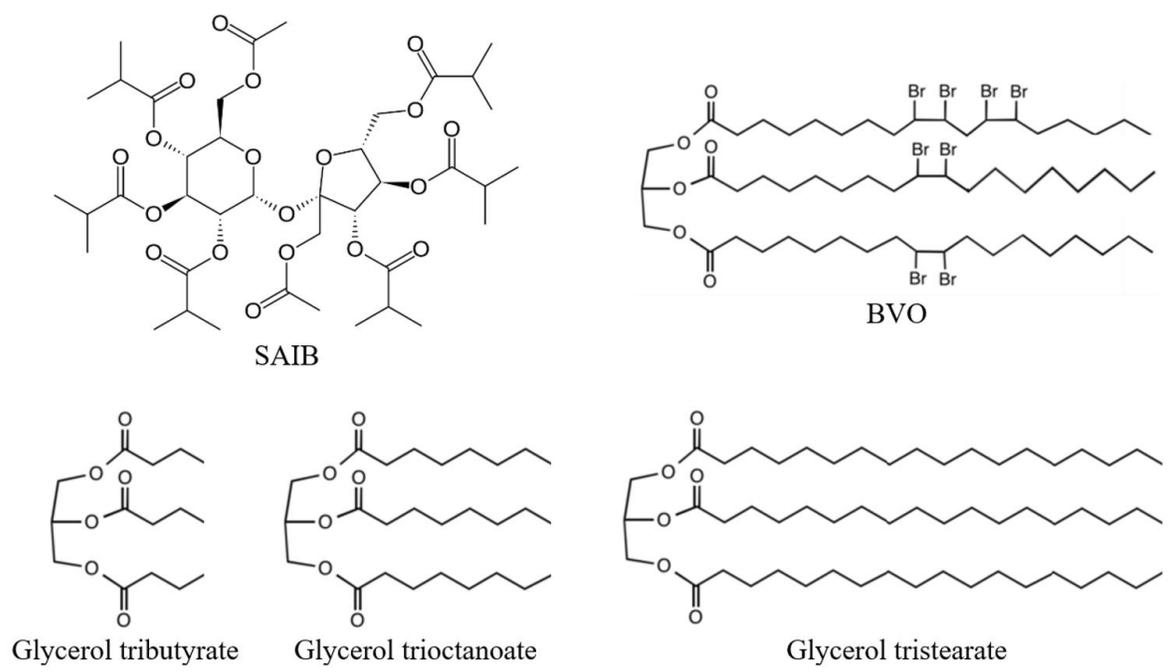


Figure 4.1. Comparison of the molecular structures of WAs SAIB and BVO and the chemical analogs utilized in this experiment Gly C<sub>4</sub>, Gly C<sub>8</sub>, Gly C<sub>18</sub>.

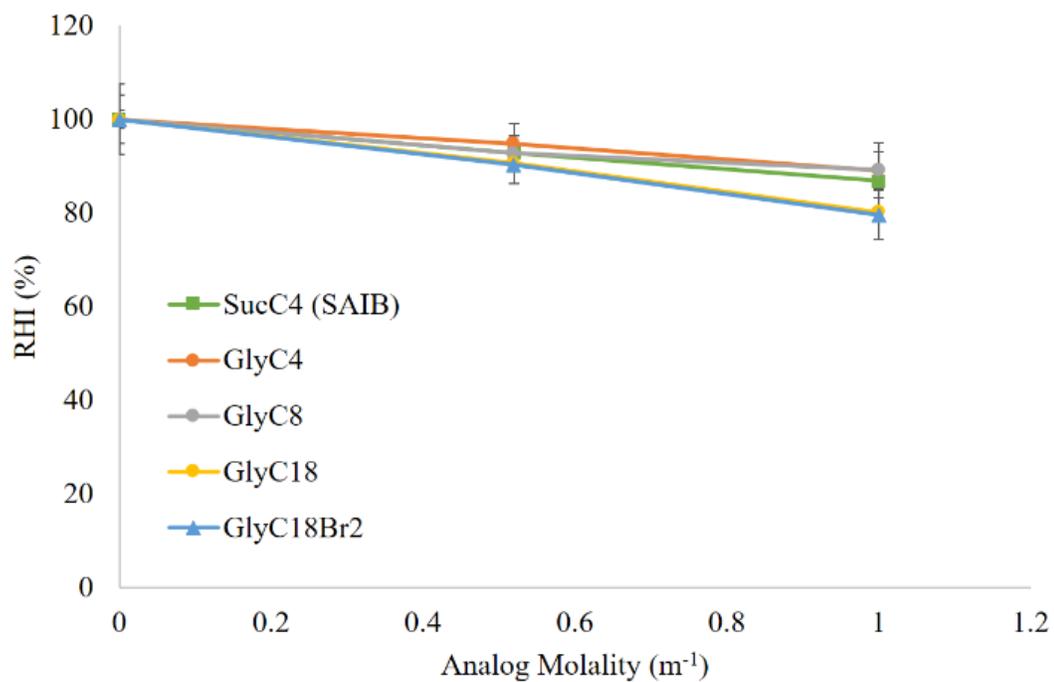
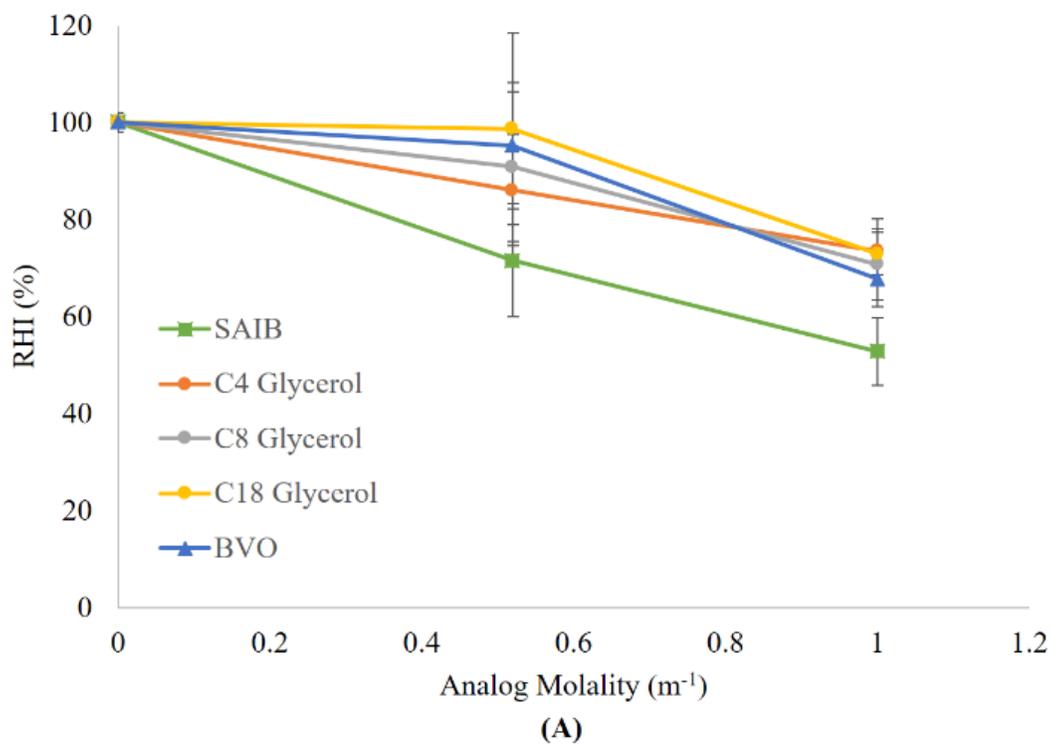
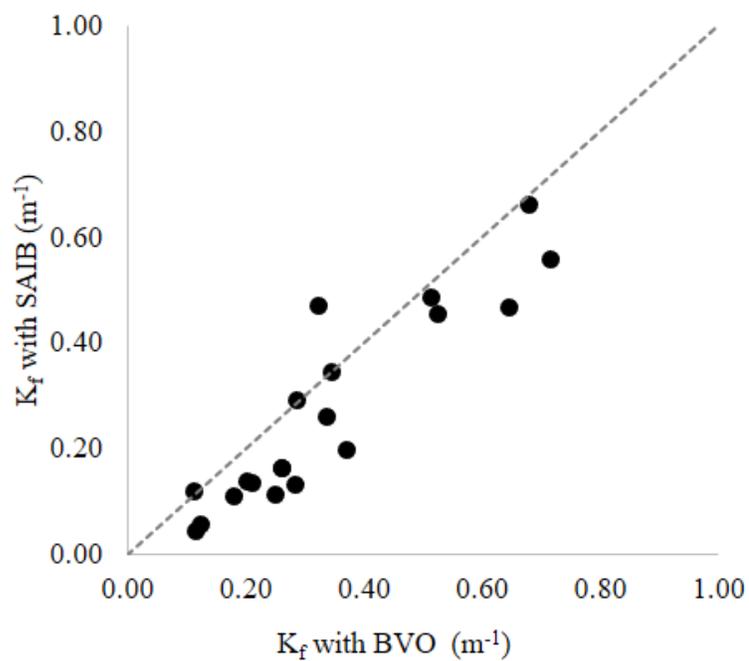
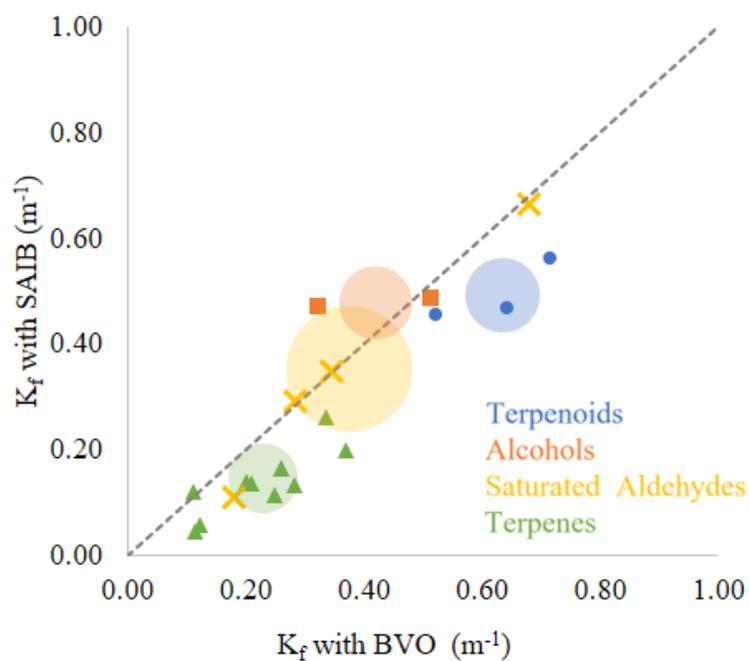


Figure 4.2. Volatility changes with increasing analog concentrations in orange oil for flavor compounds A: linalool and B:  $\beta$ -pinene. Error bars represent one standard deviation of six replicates



(A)



(B)

Figure 4.3. Comparison of the complexation of flavor compounds in orange oil to WAs SAIB and BVO for (A) each flavor compounds and (B) clusters of flavor compounds

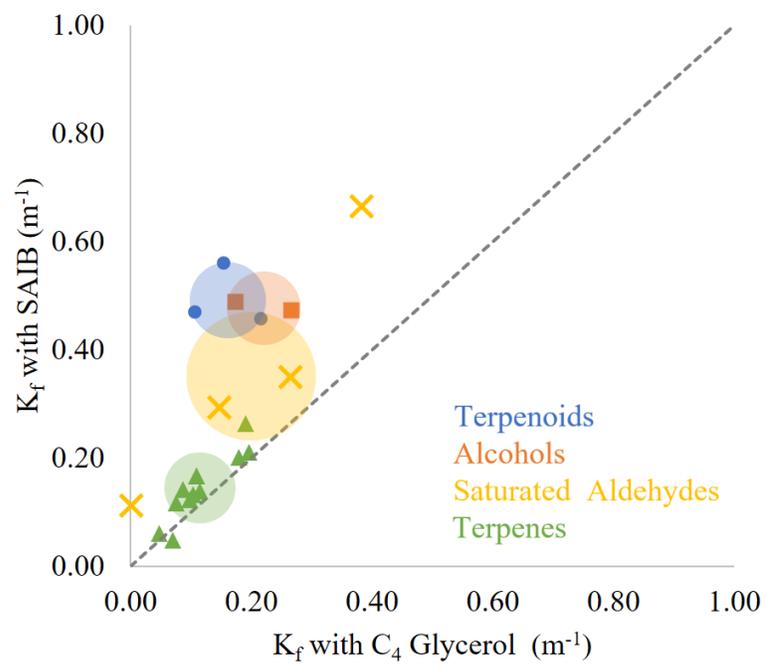


Figure 4.4. Effects of molecule backbone with a comparison of sucrose plotted against glycerol both with  $C_4$  chain length.

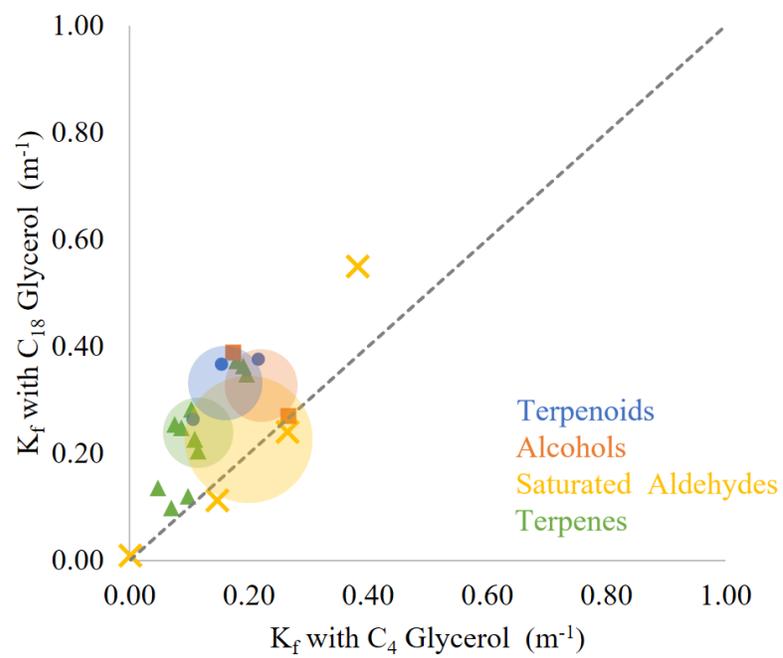


Figure 4.5. Effects of molecule chain length with a comparison of C<sub>4</sub> plotted against C<sub>18</sub> both with glycerol backbones.

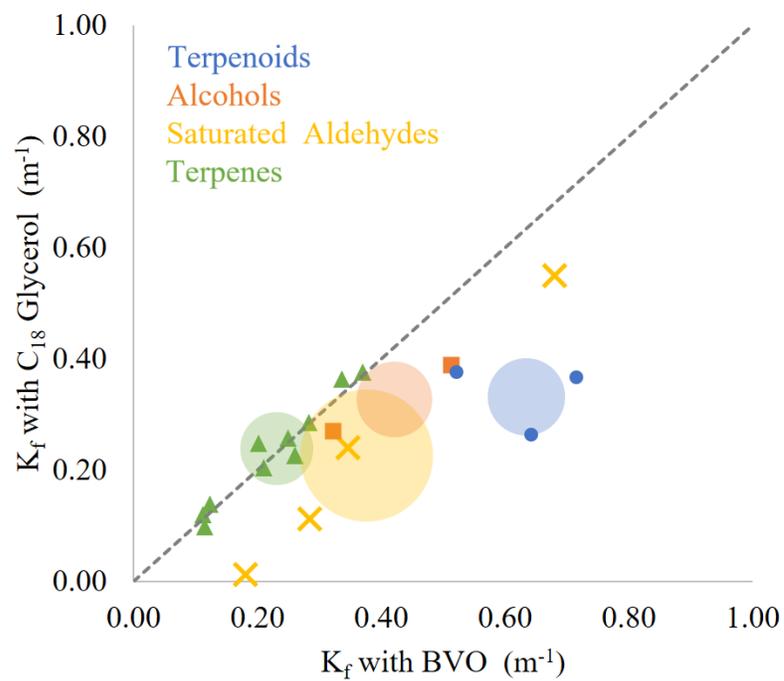


Figure 4.6. Effects of molecule bromination with a comparison of brominated vegetable oil plotted against C<sub>18</sub> non-brominated oil.

## CHAPTER 5: CONCLUSION

The addition of weighting agents (WAs) to flavor oils is commonly used by the beverage industry to stabilize flavor oil emulsions. Three of the common WAs, sucrose acetate isobutyrate (SAIB), brominated vegetable oil (BVO), and ester gum (EG), differ in both chemical compositions and required usage levels. If a WA is substituted in a beverage product, differences may impact the flavor oil blend properties and volatile compound availability. Past substitutions in beverage emulsion ingredients have demonstrated modifications to the flavor compound partitioning (Mirhosseini and Tan, 2009; Mirhosseini et al. 2008). Thus, the aim of this thesis was to assess and explain any measured changes in volatile flavor compounds of flavor oils in the presence of different WAs.

In *Hypothesis A*, it was proposed that physiochemical interactions between a WA and flavor molecule are based on flavor molecule chemistry, which selectively modifies partitioning. This hypothesis was supported by this research (Chapter 3), and intermolecular forces were suggested to modify the available concentration of certain types of flavor compounds. Selective flavor compound associations with WAs were measured and suggested to be influenced by the flavor molecule structure. Presence of carbon double bonds, alcohol groups, and aldehyde groups on a flavor compound correlated with increased associations to WAs. Within the range of molecules tested, a higher polarity and higher polarizability contributed to a greater degree of complexation across all WAs (SAIB, BVO, and EG). A model was proposed to predict association of molecules that were not studied, but may be of interest, based on molecular properties obtainable through online databases.

*Hypothesis B* theorized that differences in molecular structure of SAIB and BVO contributed to associations with flavor compounds, and thus was also supported by this

research (Chapter 4). The difference in backbone (sucrose stronger than glycerol) and bromination (presence stronger than absence) impacted the degree of association, but not chain length. Further, it was theorized that the number of excess polarizable (EP) bonds on a WA represent the number of binding sites for flavor compounds. On a basis of equal number of molecules (equal molalities), both SAIB and BVO displayed similar magnitudes in association with flavor compounds, however these WAs are different densities and thus SAIB must be used at a higher concentration. Therefore, the usage level of WA was suggested to be the differentiating factor between SAIB and BVO, due to the greater number of total binding sites in oils blends with SAIB.

Lastly, *Hypothesis C* was that miscibility of flavor compounds was impacted by different WAs and homogenization procedures. Results towards this hypothesis were not significantly different, likely due to large variations between replicates of experiments, so *Hypothesis C* was not confirmed in this thesis (Appendix A). Future work is needed to address the changes in availability of flavor compounds within aqueous systems. A stronger homogenization pressure or more emulsifier were suggested to decrease droplet size, but did not significantly impact flavor compound solubility. Significant differences were not found between flavor compounds in model beverages analyzed with sensory techniques or static solubility.

Future studies regarding the impacts of WA within flavor oils should focus on a wider range of flavor compounds and enhanced implications within a true beverage system. The flavor molecules analyzed and modeled in this experiment were all relatively hydrophobic. Flavor oil blends used in beverages contain additional compounds, with a wider range of chemistries (Hausch et al. 2015), that were not included in this work. Additionally, although

significant changes ( $p < 0.05$ ) were measured for many compounds headspace changes within the flavor oils, changes were not detectable within the flavor emulsion by GC-MS or sensory analysis. Further research should focus on reducing variability between replicates of these emulsions, and improving the sensitivity of flavor compound detection within an aqueous matrix.

This thesis is the first known analysis of flavor molecule and WA associations, providing a fundamental understanding of interactions. Knowledge of the chemical influences of both the WAs and the flavor compounds provides a powerful tool for predicting how modifications would impact the flavor oil system in a beverage. Prediction of flavor compound associations to WA, in combination with the demonstration of an adjustment by spiking pure compounds, suggested how to overcome changes when switching WAs in a formulation. Suggestion of the chemical structures within WAs that form associations with flavor compounds can guide ingredient manufacturers to match the effects of current WAs, or minimize associations, with a new WA. The principles discussed in this thesis can be utilized by ingredient companies, flavor houses, and beverages manufacturers to optimize flavor oil emulsion formulations.

## **APPENDIX A: WEIGHTING AGENT EFFECTS ON FLAVOR OIL SOLUBILITY AND VOLATILITY IN MODEL SYSTEMS AND BEVERAGES**

### **A.1. Abstract**

Although the addition of weighting agents (WAs) to beverage flavor oils is widely implemented to achieve a stable emulsion, the impacts of these agents on flavor oil compounds has not been previously published. This chapter addresses the effect of WA addition to a flavor oil, as well as, compares the common weighting agents sucrose acetate isobutyrate, brominated vegetable oil, and ester gum. Simplified systems to compare static solubility, and beverage emulsions to compare simultaneous solubility and volatility within a model system, were analyzed. Spectroscopy, gas chromatograph – mass spectroscopy, and sensory analysis were used to compare oil phase components in these systems. No statistical significance was found between weighted and unweighted oils, nor between the WAs, when considering the entire system. Large variance and poor repeatability, in addition to the initial low solubility of these flavor compounds, likely led to the lack of differentiation between samples. Additional optimization of these methods, or selection of a new flavor oil, may allow for a more accurate different ion between samples.

### **A.2. Introduction**

Beverage weighting agents (WAs) are commonly added to flavor oils in beverage emulsions to inhibit creaming (also referred to as ringing in beverage application). The high density of the WA increases the overall oil phase density and thereby reduces gravitational separation of the phases. To further aid in emulsion stability, an emulsifier and homogenization are typically used to maintain a stable droplet size. The combination of WA, emulsifier, and homogenization is needed to maintain stability of a beverage (Chanamai and

McClements, 2000). Essential flavor oils, used in citrus flavored sodas and sports drinks, are low density (approximately 0.87 g/mL), immiscible in water, and primarily composed of terpene molecules (Tan, 2004). A schematic of a beverage emulsion is shown in Figure 2.3, with the tan colored oil droplet emulsified within the blue aqueous phase. For a compound within the flavor oil to be detected as an aroma, it must be present in the headspace (Lawless and Heymann, 2010). Thus, the solubility ( $K_{OW}$ ) and volatility ( $K_{WH}$ ) partition values are two key parameters in determining the influence of a compound on flavor perception.

Past research on beverage emulsion has focused on the impacts of WAs on emulsion stability and the impacts of other components of flavor compound volatility. The effectiveness of the WAs sucrose acetate isobutyrate (SAIB), brominated vegetable oil (BVO), and ester gum (EG) to inhibit gravitational separation has been documented (Trubiano, 1995; Chanamai and McClements, 2000; Lim et al. 2011). Within model emulsions, modifications to the type or concentration of components often changes the partitioning of flavor compounds (Donsi et al. 2012; Moghimi et al. 2016). Emulsifier type and concentration has been suggested to modify flavor compound volatility in model beverage emulsions (Mirhosseini et al. 2008; Mirhosseini and Tan, 2009; Mirhosseini et al. 2010) and other model emulsions (Charles et al. 2000; Donsi et al. 2012; Cheong et al. 2014). The amount of flavor oil has also been suggested to impact volatile flavor compound availability within a flavor emulsion (Mirhosseini et al. 2008; Mirhosseini et al. 2010; Arancibia et al. 2011). Homogenization parameters, such as pressure and number of passes, has also modified the concentration of flavor compounds (Charles et al. 2000; Van Ruth et al. 2002; Chang et al. 2012; Moghimi et al. 2016). Many changes with type of concentration of

ingredients within beverage emulsions have displayed modifications to flavor compound partitioning, but no published research has analyzed the impacts of WAs.

This research aims to measure the impacts of WA on both the solubility and volatility of individual flavor compounds. In a simplified model system, with flavor oil placed on the surface of an aqueous system, spectrophotometry was used to directly measure the solubility from an oil phase into the aqueous phase and headspace (HS) gas chromatography - mass spectrometry (GC-MS) was used to concurrently measure the solubility and volatility. Model beverages were also prepared to simultaneously measure the solubility and volatility using sensory analysis (both consumer and trained panelists) as well as HS GC-MS analysis of the beverages volatiles. These analyses aimed to assess the impacts that WA usage and type played on flavor compound partitioning for applications in beverage flavor emulsions.

### **A.3. Methods and Materials**

#### **A.3.1. Sample Preparation**

Each WA (SAIB, BVO, and EG) was added to flavor oils (orange, lemon, lime, and corn (unflavored)) to create the flavor oil blends. The gum acacia (GA) was purchased from Spectrum Chemical Manufacturing Corp. (New Brunswick, NJ) and corn oil was purchased from a local supermarket. Procedures and other ingredient sources are described in detail in section 3.3.1. Each WA was added at the typical usage ratios in the oil blend (0.52 m, 0.04 m, and 0.52 m for SAIB, BVO, and EG respectively).

To form concentrated beverage emulsions (flavor emulsions), each prepared oil blend was mixed and homogenized into a GA solution. An 11.1% (w/w) GA solution was prepared 24 hours prior to homogenization by mixing the GA powder in a small amount of water, creating a paste, adding additional water to reach the allocated amount, and stirring the

solution overnight until the protein was hydrated. In a commercial blender, 10% (w/w) oil phase and 90% (w/w) GA solution were mixed, at high speed, for 30 seconds as a pre-homogenization step. This step created a coarse emulsion with 10 % GA, 10 % oil phase, and 80 % DI water (all w/w). Flavor emulsions were then homogenized at a pressure of 385 bar (350 bar at first stage and 35 bar at the second stage) with 2 passes in a GEA Niro-Soavi Panda 2K homogenizer. Model beverages were created by diluting the flavor emulsions to a final flavor oil concentration of 0.015 % (w/w) in DI water. This beverage was equilibrated 24 hours prior to sample preparation to avoid concentration gradients within the samples. Each flavor emulsion and beverage was prepared in duplicate, and each duplicate was analyzed twice, for a total of four replicates of each beverage.

#### A.3.2. UV Spectrophotometry

Static solubility of essential oil components from flavor oil blends was determined through measurement of changes in an aqueous phase absorbance. Flavor oil blends (2.5  $\mu\text{L}$ ) were added to the surface of 3.2 mL of DI water in a quartz cuvette (both at room temperature). The absorbance (between 190 nm and 450 nm) of the water phase was measured using a Shimadzu dual path spectrophotometer (Columbia, MD). A blank of pure DI water was analyzed prior to analysis. The instrument automatically recorded the absorbance every 30 minutes without physically disrupting or stirring the system.

#### A.3.3. Gas Chromatography Mass Spectrometry

Solubility of essential oil components into DI water was additionally measured by GC-MS to identify and compare the soluble components. Flavor oil blends were placed on the surface of DI water in a plastic centrifuge vial at various concentration to achieve a constant 10% (v/v) of flavor oil. After 24 hours of equilibration, a portion of water phase was

removed by piercing the plastic vial, near the bottom, to avoid contamination with the oil phase. The removed aqueous phase (100  $\mu$ L) was added to a 20 mL HS vial and analyzed using procedures detailed in section 3.3.2. Each sample was prepared and analyzed six times. Abundance of volatile compounds in model beverages were also analyzed using GC-MS. Beverage samples (1 mL) were added to 20 mL HS vials, and the remaining procedures were carried out by the auto sampler and the GC-MS, as described in section 3.3.2.

#### A.3.4. Triangle Tests

To assess consumer ability to perceive differences between model beverages, discriminatory sensory analysis was utilized. Specifically, a series of triangle tests were presented to consumers to compare SAIB, EG, and BVO weighted flavor oils (orange and lemon) within beverage systems. Serving order was blocked with all three pairs of orange flavor analyzed before the three pairs of lemon flavored beverages. Within each block, a completely randomized and balanced serving order was utilized, and all samples were given random 3-digit number codes. Panelists (n=58) were all between the ages of 18 and 65 with no reported allergies. Approximately 25 mL of each beverage was added to 2 oz plastic soufflé cups, and lids were immediately applied. Samples were prepared the morning before analysis to avoid loss of HS volatiles. Prior to analysis, this experiment received NC State University IRB approval (Number 9069)

#### A.3.5. Descriptive Analysis

Model beverages were analyzed with trained panel, descriptive analysis (DA) to measure and compare the prevalence of several attributes in each beverage. The Spectrum™ method of descriptive analysis was utilized to assess nine attributes in each beverage (aroma intensity, citrus complex, sour taste, bitter taste, astringent mouthfeel, viscosity, mouth

coating, aftertaste intensity, and bitter aftertaste). Seven trained panelists were utilized, each with a minimum of 40 hours of descriptive training in descriptive analysis (ages 22-65 years old). Panelists were instructed to evaluate the aroma intensity in the headspace prior to consumption. During consumption, citrus complex, sour taste, bitter taste, astringent mouthfeel, viscosity, and mouth coating were evaluated. Aftertaste intensity and bitter aftertaste were recorded up to a minute after expectorating, at which time the palate was cleansed before analysis of the next sample. Samples were prepared as described in section A.3.3. Prior to tasting, this experiment received NC State University IRB approval (Number 9069).

#### **A.4. Results and Discussion**

##### **A.4.1. Measurement of Solubility with Model Systems**

The static solubility of essential oil components was measured using spectrophotometry (that directly measured the aqueous phase) and GC-MS (which measured the HS above an extracted aliquot). Both methods displayed high variability and large changes between days of analysis. Likely sources of these variances are hypothesized to have been caused by inconsistency in sample preparation, such as speed or force with which the oil was added to the surface or precision of sample volumes.

Comparison of the three flavor oils (orange, lemon, and lime) showed differences in magnitudes but some similar peak absorbance wavelengths (Figure A.1). An absorbance reading of the aqueous phase after interfacing with the oil suggested the presence of two or more oil phase components that readily partition into an aqueous phase. Each of the three flavors show peaks at a wavelength of approximately 240 nm and 190 nm, indicating the presence of identical compounds in each oil (Figure A.1). The differences in magnitude

suggest the concentration of this compound is different between the types of flavor oils. Alternatively, orange oil had a maximum absorbance at 335 nm, while the other flavor oils did not. The peaks, all outside of the visible range, suggest the respective compounds are not contributing to the oil's respective colors, and may be flavor active compounds.

Impacts of WAs on the solubility of components was also analyzed, and the time curve after interfacing with an aqueous phase is shown in Figure A.2. Although high variability between replicates was observed, an overall increase in absorbance with time was readily seen. The narrowing of slope near 200-250 minutes suggest the equilibrium is not immediately reached, but instead takes time (close to 400 minutes) to reach. Graphically, the orange oil (OO, the unweighted sample) appears to produce the largest solubility, however the value was not significantly different from either the SAIB or BVO weighted samples. This method needs further optimization to reach quantitative conclusions about the rate or magnitude of solubilization of oil components. Further, identification of these compounds may be useful for practical applications to flavor oils.

In a separate experimental design, the oil components within the aqueous phase were identified with HS GC-MS. The flavor oil compounds, and the corresponding relative headspace intensity (RHI) with each WA, are displayed in Table A.1. Interestingly, a white film often appeared at the oil and water interface, although the cause or composition is unknown. Many of the components were present in levels not significantly different between the WAs ( $p > 0.05$ ), as indicated by significance lettering. Many flavor compounds displayed decreased solubility into the aqueous phase with the inclusion of any of the WAs, as evidenced by the RHI lower than 100% (Table A.1). At typical usage ratios, the RHI of linalool and terpinen-4-ol were significantly greater with BVO than with SAIB or EG as a

WA. Although this method also suffered from high variance between replicate samples, both the spectrophotometry and chromatography suggested that some oil components are soluble in the aqueous phase, and WAs tended to decrease that solubility.

#### A.4.2. Sensory Analysis of Beverage Systems

Model beverages were prepared to evaluate human perception of differences between the three WAs in a typical application. The results from each of the six comparisons (two flavors and three WAs) are displayed in Table A.2. In each case, the percent of panelists which correctly chose the different sample was not different than pure chance (1/3 at random), and thus, none of the comparisons were determined to be significantly different. Although these panelists were not able to detect a difference, it is possible these samples have only minor differences that were below detection limits. This experiment should be repeated with flavor oil blends more similar to commercial flavor blends and over different storage times, for more confidence that consumers cannot tell the difference between WAs used in beverages.

Although untrained consumers were not able to sense a difference between WAs in a beverage, trained panel DA was also used as a more sensitive test. The DA panelists tasted each beverage four times and assessed nine attributes of the beverages. The p-values corresponding to the comparison of SAIB and BVO for each of the attributes is summarized in Table A.3. Only the comparison of the viscosity between SAIB and BVO within lime and corn oil were significantly different ( $p < 0.006$ , to adjust for the nine simultaneous comparisons). Differences could have been in the true viscosity, or possibly another attribute which was perceived as viscosity. The aromatic nor taste attributes had significantly

measured differences, which was consistent with the lack of differentiation found in the triangle tests.

To compare sensory results with actual concentrations of flavor compounds, volatile composition of beverages was additionally analyzed with HS GC-MS. The percent of total peak area, for selected compounds, is displayed in Table A.4 for orange and lemon flavored beverages. Within each flavor, most of the flavor compounds were not significantly different between the three WAs.

### **A.5. Conclusions**

Neither of the sensory tests, nor headspace analysis, measured considerable differences between the beverages with different WAs, suggesting SAIB can be substituted for BVO without changes in the flavor compound solubility profile. However, additional analysis is needed to gather more information on commercial impacts of WA switches due to lack of reproducibility within this analysis. In model systems, there may have been a difference, but high variance and low repeatability (sources unknown) led to the measurement of no significant differences. Although the results from this chapter were inconclusive, more sensitive methods may be capable of detecting differences in flavor oil properties between different WAs.

## A.6. References

- Arancibia C, Jublot L, Costell E, Bayarri S. 2011. Flavor release and sensory characteristics of o/w emulsions. Influence of composition, microstructure and rheological behavior. *Food Res.Int.* 44(6):1632-41.
- Chanamai R, McClements DJ. 2000. Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J.Agric.Food Chem.* 48(11):5561-5.
- Chang Y, McLandsborough L, McClements DJ. 2012. Physical properties and antimicrobial efficacy of thyme oil nanoemulsions: influence of ripening inhibitors. *J.Agric.Food Chem.* 60(48):12056-63.
- Charles M, Rosselin V, Beck L, Sauvageot F, Guichard E. 2000. Flavor release from salad dressings: sensory and physicochemical approaches in relation with the structure. *J.Agric.Food Chem.* 48(5):1810-6.
- Cheong KW, Tan CP, Mirhosseini H, Joanne-Kam WY, Sheikh Abdul Hamid N, Osman A, Basri M. 2014. The effect of prime emulsion components as a function of equilibrium headspace concentration of soursop flavor compounds. *Chemistry Central Journal* 8(1):1-11.
- Donsì F, Annunziata M, Vincensi M, Ferrari G. 2012. Design of nanoemulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. *J.Biotechnol.* 159(4):342-350.
- Lawless HT, Heymann H. 2010. *Sensory evaluation of food: principles and practices.* 2nd ed. New York : Springer.
- Lim SS, Baik MY, Decker EA, Henson L, Michael Popplewell L, McClements DJ, Choi SJ. 2011. Stabilization of orange oil-in-water emulsions: A new role for ester gum as an Ostwald ripening inhibitor. *Food Chem.* 128(4):1023-1028.
- Mirhosseini H, Tan CP, Hamid NSA, Yusof S. 2008. Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion. *Food Chem.* 107(3):1161-72.
- Mirhosseini H, Tan CP. 2009. Response surface methodology and multivariate analysis of equilibrium headspace concentration of orange beverage emulsion as function of emulsion composition and structure. *Food Chem.* 115(1):324-333.
- Mirhosseini H, Tan CP, Kostadinovic S, Naghshineh M. 2010. Principle component analysis of equilibrium headspace concentration of beverage emulsion as function of main emulsion components. *J Food, Agriculture & Environment* 8(2):126-133.

Moghimi R, Aliahmadi A, McClements DJ, Rafati H. 2016. Investigations of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food borne pathogens. *LWT - Food Science and Technology* 71:69-76.

Tan, C. 2004. Beverage Emulsions. In: Larsson, K., & Friberg, S. *Food emulsions*. 4th ed., rev. and expanded. New York: M. Dekker. 485-524.

Trubiano PC. 1995. The Role of Specialty Food Starches in Flavor Emulsions. *ACS Symp.Ser.* 6(10):199-209.

van Ruth SM, King C, Giannouli P. 2002. Influence of lipid fraction, emulsifier fraction, and mean particle diameter of oil-in-water emulsions on the release of 20 aroma compounds. *J.Agric.Food Chem.* 50(8):2365-71.

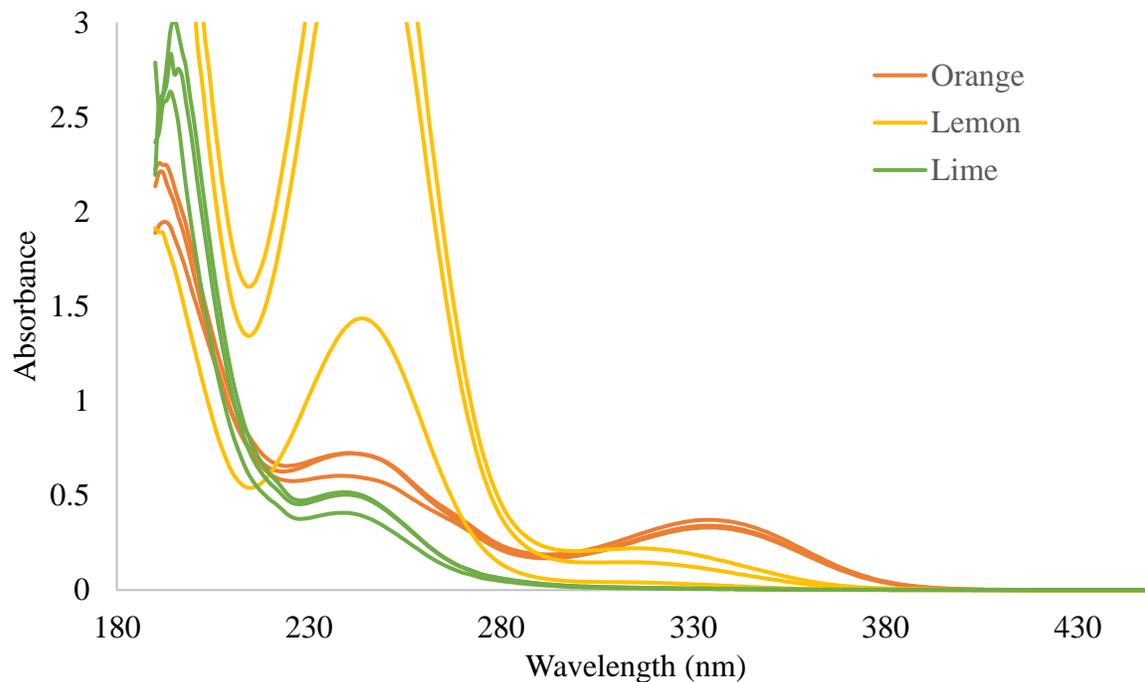


Figure A.1. Comparison of the solubility of chromophore components within orange, lemon, and lime flavor oils over the UV spectrum. Absorbance spectrums were collected from the aqueous phase after six hours of equilibration between oil and water phases.

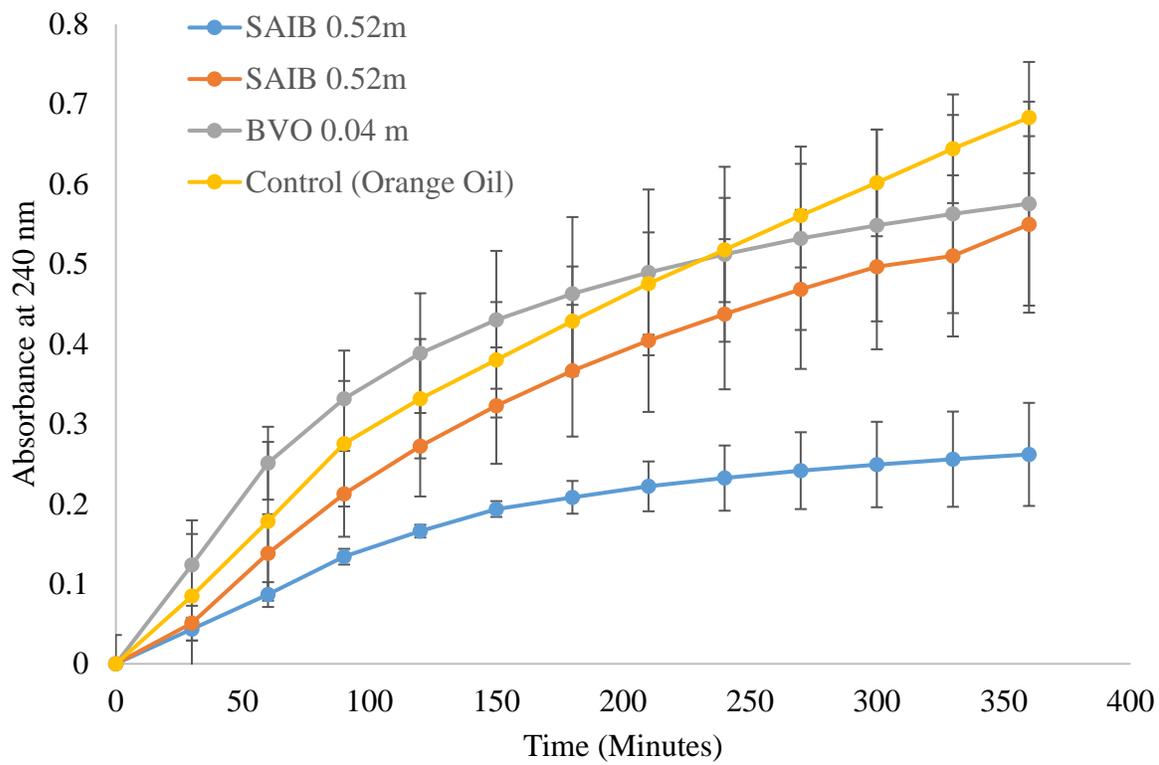


Figure A.2. Impacts of WAs on the solubility kinetics of orange oil components into an aqueous phase. Error bars represent the standard deviation of 3 samples

Table A.1. Comparison of water soluble components with the inclusion of WAs into flavor oils in a static system. Tukey LSD was used to evaluate significant differences between values.

Flavor Compound	Molality	RHI with WA (%)			
		0.52 SAIB	0.52 EG	0.52 BVO	0.04 BVO
β-Myrcene		68 <sup>a</sup>	70 <sup>a</sup>	96 <sup>a</sup>	91 <sup>a</sup>
Limonene		149 <sup>a</sup>	84 <sup>a</sup>	198 <sup>a</sup>	101 <sup>a</sup>
β-Ocimene		64 <sup>b</sup>	59 <sup>bc</sup>	85 <sup>ab</sup>	79 <sup>ab</sup>
γ-Terpinene		102 <sup>a</sup>	69 <sup>a</sup>	60 <sup>a</sup>	86 <sup>a</sup>
4-Carene		66 <sup>ab</sup>	34 <sup>b</sup>	75 <sup>a</sup>	78 <sup>a</sup>
Linalool		49 <sup>b</sup>	44 <sup>b</sup>	65 <sup>a</sup>	75 <sup>a</sup>
Nonanal		76 <sup>a</sup>	131 <sup>a</sup>	69 <sup>a</sup>	93 <sup>a</sup>
Terpinen-4-ol		65 <sup>b</sup>	55 <sup>b</sup>	67 <sup>b</sup>	80 <sup>a</sup>
α-Terpineol		57 <sup>ab</sup>	49 <sup>b</sup>	63 <sup>a</sup>	67 <sup>a</sup>
Citral (Neral)		88 <sup>ab</sup>	84 <sup>ab</sup>	127 <sup>a</sup>	57 <sup>b</sup>
Citral (Geranial)		121 <sup>ab</sup>	216 <sup>ab</sup>	245 <sup>a</sup>	49 <sup>b</sup>

\*lettering represents significant differences in flavor compound between each of the WA columns. Flavor compounds were not compared against each other.

Table A.2. Triangle tests statistics for comparisons of SAIB, BVO, and EG with orange and lemon flavor oils with n=58, corresponding to a critical value of 26 correct response for detecting a significant difference ( $p < 0.05$ ).

Flavor Oil	Statistic	WA Comparisons		
		SAIB vs. BVO	BVO vs. EG	SAIB vs. EG
Orange Oil	Percent Correct	0.38	0.36	0.33
	Number Correct (n=58)	22	21	19
	Significant? ( $N_{crit}=26$ )	No	No	No
Lemon Oil	Percent Correct	0.24	0.33	0.29
	Number Correct (n=58)	14	19	17
	Significant? ( $N_{crit}=26$ )	No	No	No

Table A.3. Overall p-values for comparisons of each measured attribute between essential oil model beverages with SAIB and BVO for 7 people and 4 replicates each (2 batch reps and 2 panel reps).

<b>Attribute</b>	<b>Flavor Oil</b>			
	<b>Orange</b>	<b>Lemon</b>	<b>Lime</b>	<b>Corn</b>
Aroma intensity	0.600	0.235	0.517	N/A
Citrus complex	0.921	0.969	0.910	N/A
Sour taste	0.844	0.623	0.748	0.585
Bitter taste	0.793	0.919	0.779	0.975
Astringent mouthfeel	0.092	0.889	0.426	0.897
Aftertaste intensity	0.727	0.639	0.745	0.841
Bitter aftertaste	0.577	0.196	0.424	0.898
Viscosity	0.237	0.157	0.000*	0.006**
Mouth coating	0.384	0.385	0.680	0.789

\* differences observed with averages of 1.4 and 1.2 for SAIB and BVO respectively

\*\* differences observed with averages of 1.1 and 1.2 for SAIB and BVO respectively

Table A.4. Percent of total peak area for volatile compounds in headspace of beverages flavored with orange and lemon oils.

Compound	Class	Percent of Total Peak Area					
		Orange Flavored			Lemon Flavored		
		SAIB	BVO	EG	SAIB	BVO	EG
Limonene	Terpene	98.1 <sup>a</sup>	98.0 <sup>a</sup>	98.1 <sup>a</sup>	55.7 <sup>a</sup>	55.5 <sup>a</sup>	54.9 <sup>a</sup>
$\alpha$ -Pinene	Terpene	0.265 <sup>a</sup>	0.250 <sup>a</sup>	0.264 <sup>a</sup>	0.378 <sup>a</sup>	0.380 <sup>a</sup>	0.374 <sup>a</sup>
$\beta$ -Pinene	Terpene	0.161 <sup>a</sup>	0.142 <sup>a</sup>	0.148 <sup>a</sup>	5.38 <sup>a</sup>	5.30 <sup>a</sup>	5.21 <sup>a</sup>
$\beta$ -Myrcene	Terpene	0.712 <sup>a</sup>	0.621 <sup>a</sup>	0.704 <sup>a</sup>	0.565 <sup>a</sup>	0.558 <sup>a</sup>	0.547 <sup>a</sup>
2-Carene	Terpene	0.007 <sup>a</sup>	0.006 <sup>a</sup>	0.010 <sup>a</sup>	0.019 <sup>a</sup>	0.020 <sup>a</sup>	0.021 <sup>a</sup>
3-Carene	Terpene	0.075 <sup>a</sup>	0.070 <sup>a</sup>	0.077 <sup>a</sup>	0.007 <sup>a</sup>	0.007 <sup>a</sup>	0.007 <sup>a</sup>
4-Carene	Terpene	0.022 <sup>a</sup>	0.018 <sup>a</sup>	0.037 <sup>a</sup>	1.25 <sup>b</sup>	1.18 <sup>b</sup>	1.55 <sup>a</sup>
Camphene	Terpene	0.003 <sup>a</sup>	0.002 <sup>a</sup>	0.002 <sup>a</sup>	0.017 <sup>a</sup>	0.018 <sup>a</sup>	0.017 <sup>a</sup>
$\beta$ -Ocimene	Terpene	0.011 <sup>a</sup>	0.009 <sup>a</sup>	0.010 <sup>a</sup>	0.108 <sup>a</sup>	0.107 <sup>a</sup>	0.103 <sup>a</sup>
$\gamma$ -Terpinene	Terpene	0.017 <sup>a</sup>	0.016 <sup>a</sup>	0.021 <sup>a</sup>	30.5 <sup>a</sup>	30.0 <sup>b</sup>	28.9 <sup>c</sup>
Linalool	Alcohol	0.276 <sup>a</sup>	0.315 <sup>a</sup>	0.280 <sup>a</sup>	0.031 <sup>b</sup>	0.036 <sup>a</sup>	0.033 <sup>ab</sup>
$\alpha$ -Terpineol	Alcohol	0.006 <sup>a</sup>	0.006 <sup>a</sup>	0.006 <sup>a</sup>	0.024 <sup>b</sup>	0.027 <sup>a</sup>	0.025 <sup>ab</sup>
Hexanal	Aldehyde	0.005 <sup>a</sup>	0.004 <sup>a</sup>	0.004 <sup>a</sup>	0.006 <sup>a</sup>	0.006 <sup>a</sup>	0.007 <sup>a</sup>
Octanal	Aldehyde	0.005 <sup>a</sup>	0.012 <sup>a</sup>	0.003 <sup>a</sup>	0.019 <sup>a</sup>	0.020 <sup>a</sup>	0.021 <sup>a</sup>
Nonanal	Aldehyde	0.019 <sup>ab</sup>	0.030 <sup>a</sup>	0.014 <sup>b</sup>	0.013 <sup>a</sup>	0.015 <sup>a</sup>	0.024 <sup>a</sup>
Decanal	Aldehyde	0.143 <sup>a</sup>	0.241 <sup>a</sup>	0.150 <sup>a</sup>	0.009 <sup>a</sup>	0.009 <sup>a</sup>	0.015 <sup>a</sup>
Citronellal	Terpenoid	0.012 <sup>a</sup>	0.016 <sup>a</sup>	0.012 <sup>a</sup>	0.013 <sup>a</sup>	0.015 <sup>a</sup>	0.013 <sup>a</sup>
Citral (Neral)	Terpenoid	0.004 <sup>a</sup>	0.005 <sup>a</sup>	0.004 <sup>a</sup>	0.042 <sup>a</sup>	0.050 <sup>a</sup>	0.045 <sup>a</sup>
Citral (Geranial)	Terpenoid	0.006 <sup>a</sup>	0.009 <sup>a</sup>	0.006 <sup>a</sup>	0.093 <sup>a</sup>	0.112 <sup>a</sup>	0.104 <sup>a</sup>

\*Different letters indicate significantly different values, when comparing each volatile compound within each flavor separately.

## APPENDIX B: SUPPLEMENTAL MATERIAL

Table B.1. Volatile compounds RHI (%) with inclusion of SAIB, BVO, and EG at concentrations of 0.52, 0.28, 0.15, and 0.04 molality in orange oil flavor blends.

WA Molality	SAIB				EG				BVO			
	0.52	0.28	0.15	0.04	0.52	0.28	0.15	0.04	0.52	0.28	0.15	0.04
$\alpha$ -Pinene	93	96	96	97	93	95	95	97	90	94	96	96
$\beta$ -Pinene	88	93	94	93	93	94	93	95	86	92	95	95
$\beta$ -Myrcene	90	96	95	95	98	97	97	97	92	95	97	96
2-Carene	93	99	94	100	110	107	110	102	88	84	95	94
3-Carene	94	98	96	95	96	95	96	97	90	95	97	95
4-Carene	91	105	91	99	105	96	102	101	86	83	95	98
Camphene	83	87	90	92	91	90	91	94	78	87	89	86
$\beta$ -Ocimene	82	105	93	85	98	98	100	91	81	93	98	90
$\gamma$ -Terpinene	101	111	98	118	113	113	135	117	94	101	95	106
Linalool	66	82	83	87	86	83	92	93	84	91	93	91
$\alpha$ -Terpineol	64	85	77	77	77	73	90	90	73	88	88	81
Hexanal	67	79	86	89	80	85	88	92	74	91	94	89
Octanal	78	87	90	95	73	81	88	96	78	83	90	95
Nonanal	77	93	87	89	76	77	86	93	80	87	91	92
Decanal	82	101	81	81	59	76	83	89	86	90	91	86
Citronellal	47	85	73	57	76	64	53	72	62	70	87	81
Citral (Neral)	42	88	64	51	86	58	75	63	32	70	76	70
Citral (Geranial)	49	84	62	56	91	68	72	72	53	70	74	65

Table B.2. Molecular properties for each volatile compound utilized as possible parameters in model selection.

<b>Compound</b>	<b>K<sub>r</sub></b>	<b>Log P</b>	<b>A</b>	<b>TPSA</b>	<b>Molar Volume</b>	<b>TPSA per Volume</b>	<b>Polarizability</b>	<b>Log K<sub>oa</sub></b>	<b>Log K<sub>aw</sub></b>	<b>Log K<sub>ow</sub></b>	<b># rings</b>	<b>Percent of Oil</b>
$\alpha$ -Pinene	0.10	4.37	44.0	0.00	155	0.0	17.40	3.36	1.08	4.48	2	1.120
$\beta$ -Pinene	0.17	4.37	43.8	0.00	153	0.0	17.30	3.34	0.82	4.16	2	0.781
$\beta$ -Myrcene	0.14	4.58	47.6	0.00	177	0.0	18.90	3.75	0.42	4.17	0	2.333
2-Carene	0.11	4.37	44.0	0.00	155	0.0	17.40	3.74	0.64	4.38	1	0.053
3-Carene	0.06	4.37	44.0	0.00	155	0.0	17.40	3.74	0.64	4.38	1	0.159
4-Carene	0.12	4.27	44.0	0.00	157	0.0	17.40	3.74	0.64	4.38	1	0.046
Camphene	0.27	4.37	43.8	0.00	153	0.0	17.30	3.40	0.82	4.22	2	0.005
$\beta$ -Ocimene	0.14	4.70	47.9	0.00	176	0.0	19.00	3.40	1.40	4.80	0	0.026
$\gamma$ -Terpinene	0.10	4.36	45.4	0.00	161	0.0	18.00	4.54	-0.04	4.50	1	0.021
Linalool	0.53	3.28	49.5	20.2	180	11.5	19.60	6.03	-3.06	2.97	0	0.650
$\alpha$ -Terpineol	0.44	2.79	47.1	20.2	165	11.9	18.70	6.58	-3.30	2.98	1	0.010
Hexanal	0.55	1.97	30.0	17.1	125	14.8	11.90	4.41	-2.06	1.78	0	0.011
Octanal	0.39	3.03	39.3	17.1	158	11.5	15.60	5.36	-1.68	2.78	0	0.207
Nonanal	0.31	3.56	43.9	17.1	174	10.3	17.40	4.79	-1.52	3.27	0	0.051
Decanal	0.17	4.09	48.6	17.1	191	9.3	19.30	4.89	-1.13	3.76	0	0.157
Citronellal	0.53	3.48	48.4	17.1	185	9.7	19.20	5.39	-1.56	3.83	0	0.021
Citral (Neral)	0.53	3.17	48.4	17.1	178	10.1	19.20	5.26	-1.81	3.45	0	0.009
Citral (Geranial)	0.46	3.17	48.4	17.1	178	10.1	19.20	5.26	-1.81	3.45	0	0.011

Table B.3. Correlation ( $R^2$ ) of the chosen variables with the predicted value ( $K_f$ ) and each non-transformed variable utilized as a possible parameter in model selection.

<b>Parameter</b>	<b><math>K_f</math> with SAIB</b>	<b>Log P</b>	<b>A</b>
$K_f$ with SAIB	1.000	0.772	0.009
Log P	0.772	1.000	0.174
A	0.009	0.174	1.000
TPSA	0.730	0.715	0.000
Molar volume	0.014	0.031	0.763
TPSA per volume	0.765	0.848	0.029
Polarizability	0.008	0.167	1.000
Log Koa	0.560	0.519	0.054
Log Kaw	0.725	0.754	0.000
Log Kow	0.652	0.907	0.238
Number of rings	0.260	0.213	0.010
Percent of peak area in orange oil	0.080	0.114	0.021