

IDENTIFICATION AND CHARACTERIZATION OF POTENT ODORANTS  
RESPONSIBLE FOR TYPICAL AND STORAGE-INDUCED FLAVORS OF  
COLA-FLAVORED CARBONATED BEVERAGES

BY

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DISSERTATION

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## ABSTRACT

Cola is the predominant flavor among the various types of carbonated beverages. The uniqueness and complexity of cola flavor are the result of natural flavor ingredients, such as essential oils and vanilla extract. Many of the volatile components of these flavorings, however, are not stable under acidic conditions due to acid-catalyzed reactions. Degradation of typical potent odorants and formation of storage-induced odorants by these reactions leading to flavor changes in cola-flavored carbonated beverages were evaluated in the present study.

The potent odorants in the top three US brands of regular colas were characterized by aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry (GCO), and GC-mass spectrometry (GC-MS). Among the numerous odorants, eugenol and coumarin were predominant in all regular colas. Guaiacol and linalool were predominant odorants in at least one brand, while 1,8-cineol was a moderately potent odorant in all colas. In addition, aroma profiles determined by sensory descriptive analysis were in good agreement with the potent odorants identified by AEDA.

The typical potent odorants identified by AEDA were verified by sensory evaluation of an aroma reconstitution model, and the character-impact odorants of typical cola were evaluated by omission studies. Thirty typical odorants were accurately quantified by stable isotope dilution analysis (SIDA), and their odor activity values (OAVs) were calculated as the ratios of their concentration to their odor detection thresholds in water. The OAV results, in which enantiomeric distribution was taken into account, revealed that 1,8-cineol, (*R*)-(-)-linalool, and octanal made the greatest contribution to the overall aroma of cola, followed by nonanal, (*S*)-(+)-linalool, decanal, and (*R*)-(+)-limonene. The aroma reconstitution model was then constructed

based on quantification data by adding twenty high purity standards to an aqueous sucrose-phosphoric acid solution. Headspace-solid phase microextraction (HS-SPME) and additional sensory analyses were used to adjust the model to better match authentic cola.

The aroma profile of the re-balanced aroma reconstitution model did not differ from that of authentic cola and, thus, it was used as a complete model for omission studies. The omission models were prepared by omitting groups of odorants, and their aromas were compared to the complete model by sensory evaluation (R-index ranking test). The results indicated that aroma of one omission model, composed of methyleugenol, (*E*)-cinnamaldehyde, eugenol, and (*Z*)- and (*E*)-isoeugenols, differed from the complete model. However, the omission models for the individual components of this group did not differ from the complete model. These results indicate that there is no single character-impact aroma compound in cola, and instead a balance of numerous odorants is responsible for the characteristic aroma of cola-flavored carbonated beverages.

Aroma changes in cola stored at various temperatures (5°C, room temperature, and 40°C) for three months were investigated by sensory and instrumental analysis. The results of R-index ranking test and sensory descriptive analysis revealed that the aroma of cola stored at 40°C was significantly different from that of typical cola. The potent odorants of stored colas were also characterized by AEDA. Eugenol and coumarin were the most potent odorants in all stored colas. The potency of octanal and linalool declined as a function of increasing storage temperature. Additionally, *p*-cresol was identified as a potential off-flavor in temperature-abused (40°C) cola. These results were in good agreement with the relative concentrations determined by GC-MS. Some typical potent odorants were not stable during storage due to temperature dependent acid-catalyzed reactions. In addition, 2-carene and two unidentified volatiles could be used as the chemical markers to indicate high temperature storage abuse.

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## ABBREVIATIONS USED

3-AFC	3-alternative force choice
AEDA	aroma extract dilution analysis
ANOVA	analysis of variance
BET	best estimate threshold
CLLE	continuous liquid-liquid extraction
DHA	dynamic headspace analysis
EI	electron-impact
FD	flavor dilution
FID	flame ionization detector
GC-MS	gas chromatography-mass spectrometry
GCO	gas chromatography-olfactometry
HS	headspace
LSD	least significant difference
NMR	nuclear magnetic resonance
OAV	odor activity value
R <sub>f</sub>	response factor
RI	retention index
RT	retention time
SAFE	solvent assisted flavor evaporation
SIDA	stable isotope dilution analysis
SPME	solid phase microextraction
UHP	ultra high purity

# CHAPTER 1

## INTRODUCTION

Carbonated beverages represent the largest segment of the soft drink industry. Cola is the predominant flavor among the various types of commercial carbonated beverages, and accounts for 54.0% of the global revenues generated by the soft drink and bottled water industry (IBISWorld, 2011). In 2010, the sales of cola-flavored carbonated beverages in the United States made up 30% of the soft drink market or about \$14 billion (Kaczanowska, 2010a, 2010b). Despite its popularity, there is limited information about the flavor chemistry of cola. In fact, the recipe of Coca-Cola is the one of the world's most famous trade secrets. The major manufacturers of commercial colas list natural flavors on the ingredient labels. Cola flavorings may contain vanilla extract and essential oils of citrus, cassia or cinnamon, coriander, nutmeg, and neroli (Tchudi, 1986; Pendergrast, 2000). The mixture of these ingredients and some other unknown (trade secret) ingredients are responsible for the uniqueness and complexity of the product, and produce the typical and highly familiar aroma of cola.

As mentioned above, only a few studies have been conducted on cola-flavored carbonated beverages. This is surprising considering the popularity of cola flavor, especially since other popular beverages (e.g., tea, coffee, fruit juices, wine, etc.) have received far more attention. Sensory attributes of cola, including aroma, aroma-by-mouth, mouthfeel, taste, and afterfeel, were evaluated by descriptive analysis (Kappes et al., 2006). The aroma volatiles of cola have been studied by instrumental analysis as well. Cola was chosen as a sample to compare various methods for the analysis of headspace volatiles (Elmore et al., 1997). The results of that study indicated that terpenes and aldehydes were the most abundant volatile

compounds in colas. The unique volatiles of cola and cola stain were also studied along with the other dark color beverages for criminological purposes (Hida et al., 1998).

While cola flavor is highly desirable, it is, unfortunately, unstable due to the acid-catalyzed degradation of certain volatile components of the essential oils used in the flavorings. In particular, terpenoids, which are the major volatiles in regular and diet colas (Elmore et al., 1997), are generally unstable in acidic solution (Baxter et al., 1978; Cori et al., 1986; Schieberle and Grosch, 1988). This is due to acid-catalyzed hydration, dehydration, rearrangement, cyclization, and hydrolysis reactions (Clark and Chamblee, 1992). *p*-Cymene, possibly formed as dehydrogenation product of  $\gamma$ -terpinene or limonene from lemon oil, was reported to cause an off-odor in cola due to its accumulation during storage and its undesirable odor characteristics (Wiley et al., 1984). In addition to *p*-cymene, several other compounds can cause off-notes in lemon oil containing products. These include 2-*p*-tolyl-propene (Kimura et al., 1982), *p*-cresol and *p*-methylacetophenone (Schieberle and Grosch, 1988). Moreover,  $\alpha$ -*p*-dimethylstyrene and *p*-cymen-8-ol were also reported as off-flavors in a carbonated citral-containing beverage (Peacock and Kuneman, 1985). It is possible that these compounds may be more potent off-flavor compounds than *p*-cymene in stored cola.

Flavor changes in stored cola may be due to degradation of potent odorants and/or caused by the formation of off-odors as a result of acid-catalyzed reactions. Both types of reactions may lead to a reduction in product quality. There is lack of information about deterioration of potent odorants and development of storage-induced odorants in cola.

The long term goal of the present research is to characterize changes in the aroma components of stored cola-flavored carbonated beverages by acid-catalyzed reactions. The central hypothesis of this study is that aroma changes in stored colas are caused by both the

formation of storage-induced off-odorants and by the degradation of desirable odorants as a result of acid-catalyzed reactions. Three specific aims on the basis of flavor analysis and sensory evaluation were tested to achieve the long term goal of this project.

For the first specific aim in **Chapter 3**, the aroma compounds in typical cola were characterized using gas chromatography-olfactometry (GCO) and GC-mass spectrometry (GC-MS). The potent odorants in the top three US brands of regular colas were identified by aroma extract dilution analysis (AEDA), and based on the assumption that the compounds having the highest flavor dilution (FD) factors are the potent odorants.

The potent odorants in cola were further evaluated by calculation of odor active values (OAVs) and by omission studies in **Chapter 4**. The hypothesis of this specific aim two was that cola-flavored carbonated beverages contain character-impact odorants that must be present in order for the product to have its typical aroma. Thus, omission of a character-impact odorant will cause a detectable change to occur in the overall aroma quality and/or intensity of the product.

The aroma changes in stored cola were investigated in **Chapter 5**. Causes of flavor changes, including formation of storage-induced odorants and/or deterioration/loss of typical odorants, were established. The odorants which are responsible for the flavor of stored cola were indicated using the analytical data. It was hypothesized that storage results in the formation and/or loss of specific and predictable flavor (aroma) compound in cola-flavored carbonated beverages by acid-catalyzed reactions.

This is the first study in which flavor chemistry of cola was established. The potent odorants and character-impact odorants of cola-flavored carbonated beverages were characterized using advanced flavor science techniques, such as stable isotope dilution analysis

and aroma reconstitution models. The novel and convenient methods to synthesize the specific deuterium-labeled standards were also developed for the accurate quantification of aroma compounds in this work. In addition, this study establishes for the first time by use of sensory and instrumental analyses the potent odorants responsible for storage-induced off-flavors in cola. *p*-Cresol is reported for the first time as an off-odorant in storage temperature-abused cola, and 2-carene and two other unidentified volatiles were established as unique chemical markers to indicate temperature abuse during storage. The present study expands the knowledge of the flavor chemistry of cola-flavored beverages, which can lead to improvements in the flavor quality, flavor stability and shelf-life of this important and popular carbonated beverage.

## REFERENCES

- Baxter, R.L.; Laurie, W.A.; McHale, D. Transformations of monoterpenoids in aqueous acids: The reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid. *Tetrahedron* **1978**, *34*, 2195-2199.
- Clark, B.C., Jr.; Chamblee, T.S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, 1992; pp 229-285.
- Cori, O.; Chayet, L.; Perez, L.M.; Bunton, C.A.; Hachey, D. Rearrangement of linalool, geraniol, and nerol and their derivatives. *J. Org. Chem.* **1986**, *51*, 1310-1316.
- Elmore, J.S.; Erbahadir, M.A.; Mottram, D.S. Comparison of dynamic headspace concentration on Tenax with solid phase microextraction for the analysis of aroma volatiles. *J. Agric. Food Chem.* **1997**, *45*, 2638-2641.
- Hida, Y.; Matsumoto, M.; Kudo, K.; Imamura, T.; Ikeda, N. Identification of beverages and beverage stains by GC/MS using aroma components as indicators. *Int. J. Legal. Med.* **1998**, *111*, 13-16.
- IBISWorld. Global Soft Drink & Bottled Water Manufacturing. *Global Industry Report C1124-GL* [Online], November 9, 2011, p 14. IBISWorld.  
<http://clients.ibisworld.com/globalindustry/default.aspx?indid=420> (accessed January 12, 2012).
- Kaczanowska, A. Carbonated soft drink production in the US. *IBISWorld Industry Report 31211a* [Online], December, 2010b, p 12. IBISWorld.  
<http://www.ibisworld.com/industryus/default.aspx?indid=285> (accessed December 9, 2010).
- Kaczanowska, A. Soft drink production in the US. *IBISWorld Industry Report 31211* [Online], August, 2010a, pp 13-14. IBISWorld.  
<http://www.ibisworld.com/industryus/default.aspx?indid=284> (accessed December 9, 2010).
- Kappes, S.M.; Schmidt, S.J.; Lee, S-Y. Descriptive analysis of cola and lemon/lime carbonated beverages. *J. Food Sci.* **2006**, *71*, S583-S589.
- Kimura, K.; Iwata, I.; Nishimura, H. Relationship between acid-catalyzed cyclization of citral and deterioration of lemon flavor. *Agric. Biol. Chem.* **1982**, *46*, 1387-1389.
- Peacock, V.E.; Kuneman, D.W. Inhibition of the formation of  $\alpha$ -*p*-dimethylstyrene and *p*-cymen-8-ol in a carbonated citral-containing beverage system. *J. Agric. Food Chem.* **1985**, *33*, 330-335.

Pendergrast, M. *For God, Country and Coca-Cola*; Basic Books: New York, 2000; pp 456-460.

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

Tchudi, S.N. *Soda Poppery*; Charles Scribner's sons: New York, 1986; pp 32-34.

Wiley, R.C.; Louie, M.K.; Sheu, M-J. Studies on turpentine-like off-odor in cola. *J. Food Sci.* **1984**, *49*, 485-488.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **I. HISTORY OF COLA**

The universally recognized flavor we know as “cola” was not developed until well after techniques for the practical carbonation of water had been developed and already in use for production of other commercial carbonated beverages. The history of carbonated beverages is recorded in several sources (Tchudi, 1986; Hargitt, 2006). Carbon dioxide was discovered by European scientists in the middle of 1700s, and was used to produce artificial mineral water in spas. Carbonated water was prepared using bicarbonate salts in 1741 by William Brownrigg, and it was sold as an illness remedy by Richard Bewley in 1767. By the late 1760s carbon dioxide was successfully dissolved into water under pressure by Dr. Joseph Priestley (British scientist). Carbonation equipment was later developed by Dr. John Mervin Nooth in 1775. The first commercial manufacture of carbonated water was done in Manchester (UK) by Thomas Henry in 1781, and later there were many carbonation plants in Europe. Commercial carbonation using high pressure carbon dioxide gas was later developed by Nicholas Paul at the beginning of the 1800s. Carbonated water was introduced to America before 1800, and Benjamin Silliman (a chemistry professor at Yale University) and his partner founded the first commercial plant in Newhaven, CT in 1807. Joseph Hawkins also established a plant in Philadelphia at about the same time period.

Carbonated beverages have been flavored since around 1850. In the beginning, carbonated beverages which were sold in American drugstores were infused with roots and/or herbs for medical purposes. In 1886, Dr. John S. Pemberton (American pharmacist) mixed cola

(or kola), which was a popular ingredient in Scotland and London, with coca leaf extract. Sugar and lemon oil were also added to mask the bitter taste of the caffeine from the cola nut. Later, Pemberton founded the Coca-Cola Company in Atlanta, Georgia. The company was sold to Asa G. Candler in 1892, and the cola-flavored carbonated beverage has been advertised and sold as a refreshing drink.

## **II. COLA PRODUCTION**

The production of cola-flavored carbonated beverage starts from water which is the main ingredient. Raw water is first purified by several steps before production, which is reviewed by Tatlock (2006). Sand filtration is used first to remove any particles. Water is further passed through a carbon filter to remove any organic and aroma components, and then passed through a two  $\mu\text{m}$  filter to remove cryptosporidium. Physical and chemical treatments are the next steps to improve the quality of water to achieve the desirable standards; for example, alkalinity reduction using hydrating lime, nitrate removal by ion exchange, and chlorination to eliminate any pathogens. Filtration through carbon is usually used to remove the chlorine residue after the chlorination process. In addition, other purification methods may be applied, such as ultraviolet (UV) light, ultrafiltration, nanofiltration, or reverse osmosis. The purified water is further de-aerated to minimize the oxidation reactions which could cause undesirable flavors in the finished product. The de-aeration process can be performed by vacuum and/or by refluxing to obtain water which contains less than 0.5 ppm of oxygen.

The next unit operation is accomplished in the syrup room (Horman, 2006). Sugar or other sweetener, acid, and pre-mix (other minor ingredients) are prepared individually in the mixing tank since some ingredients require warm water to dissolve. This step is also allows the

operator to check the quality before mixing with the main ingredients. The flavoring which is called concentrate is added to the main mixing tank along with syrup and de-aerated water in a closed system to avoid absorption of oxygen. This liquid product is further flash pasteurized before carbonation.

The liquid product is usually carbonated at refrigeration temperature around 4°C (Steen, 2006). There are two carbonation methods. The “active” technique is done by injection and dispersion of carbon dioxide into the liquid product. Alternatively, carbonation can be accomplished by spraying the liquid product into a carbon dioxide rich atmosphere. In either case, the carbonated beverage is immediately filled into pre-washed bottles or cans.

### **III. COLA FLAVOR**

Commercial cola manufacturers list “natural flavors” on their ingredient labels. Cola flavorings may contain essential oils and vanilla extract (Tchudi, 1986; Pendergrast, 2000). The combination of these ingredients and some other unknown (trade secret) ingredients are responsible for the uniqueness and complexity of cola flavor, and produce the typical and highly familiar aroma of cola. However, despite its popularity, there is limited research and published literature on the flavor chemistry of cola.

Aroma of cola-flavored carbonated beverage has been studied by both sensory (Kappes et al., 2006) and instrumental (Wiley et al., 1984; Elmore et al., 1997; Hida et al., 1998) techniques. Citrus, caramel, and vanilla were used as aroma attributes, along with other taste and mouthfeel attributes, to rate the flavor of cola by sensory descriptive analysis (Kappes et al., 2006). The volatile compounds of de-carbonated colas were determined by two headspace techniques, including solid phase microextraction (SPME) and dynamic headspace analysis

(DHA) (Elmore et al., 1997). They found that terpenes and aldehydes were the major components in regular and diet colas, and limonene and a mixture of  $\alpha$ - and  $\gamma$ -terpineol were present at high concentrations. The volatiles found in the two colas analyzed were similar, but the total quantity of volatiles in the regular cola was four times higher than in the diet drink. A comparison of the two extraction techniques was also included in this article.

The presence of a terpene-like off-odor in cola was studied by Wiley et al. (1984) using a headspace technique. The top three most abundant volatiles in colas were identified as limonene,  $\gamma$ -terpinene, and *p*-cymene. *p*-Cymene increased over the course of the shelf-life study, while the other two volatiles decreased. Since the increase in *p*-cymene correlated well with the degree of off-odor measured by sensory evaluation, it was concluded that accumulation of *p*-cymene resulted in the off-odor development. They also stated that *p*-cymene was the dehydrogenation product of  $\gamma$ -terpinene and/or limonene, and the titanium dioxide in the closure liner of the cola bottle acted as a catalyst of this reaction. Meanwhile, the use BHT at 750 ppm in the closure liner decreased the formation of *p*-cymene.

Another study on aroma compounds in cola was aimed at identifying unique volatiles in various dark colored beverages and beverage stains, which could be used to discriminate the various beverages for criminological purposes (Hida et al., 1998). The volatile compounds were isolated by solid phase extraction before analysis by gas chromatography-mass spectrometry (GC-MS). Among the six volatile compounds detected the cola stains, (*Z*)-terpin hydrate was reported as being unique to cola.

#### **IV. NATURAL FLAVORS USED IN COLA**

The aroma compounds in cola-flavored carbonated beverages are derived mainly from ingredients, such as vanilla extract, lime juice, and essential oils of orange, lemon, cassia or cinnamon, coriander, nutmeg, and neroli (Tchudi, 1986; Pendergrast, 2000). According to the labels on commercial colas, it is possible that lime juice has been replaced by lime oil. The major composition of each flavoring is reviewed below. It should be noted that the characteristic aroma of a flavoring may not necessarily be due to the most abundant volatile compounds. The main constituents found in essential oils are usually quantified for quality control purposes. It should be also noted that compounds found in each flavoring are usually present in varying proportions. The factors which influence these variations include cultivar (Mitiku et al., 2000), origin (Ranadive, 1992; Simpson and Jackson, 2002), season (Staroscik and Wilson, 1982), part of material (Kaul et al., 2003), and extraction method (Ferhat et al., 2007).

##### **Citrus Oils**

These include oils of lemon, lime, and orange which are usually obtained by cold-pressing technique. Limonene, a monoterpene hydrocarbon, is the most abundant volatile component of lemon oil (Coppella and Barton, 1987; Njoroge et al., 1994). The major alcohol and aldehyde are  $\alpha$ -terpineol and citral (mixture of neral and geranial), respectively (Chamblee et al., 1991). Among the numerous volatiles found in lemon oil, geranial, neral, and linalool are the most odor-active compounds which contribute to the overall flavor of lemon oil (Schieberle and Grosch, 1988).

Limonene is also the main volatile component of lime peel oil (Minh et al., 2002) and orange oil (Högnadóttir and Rouseff, 2003). A dominant alcohol in orange oil is linalool

(Sawamura et al., 2005), and it is perceived at highest intensity next to octanal and wine lactone (Högnadóttir and Rouseff, 2003). The most potent odorants found in orange peel oil are linalool, myrcene, limonene, and 1,8-cineol (Fischer et al., 2008).

Terpenes, which make up the majority of the volatile compounds of citrus essential oil, are not stable even during distillation processes. They are decomposed by acid-catalyzed reactions to form undesirable flavors such as “processed” odors. The acid-catalyzed reactions of terpenes including cyclization, hydration, dehydration, rearrangement, and hydrolysis of esters have been reviewed (Clark and Chamblee, 1992). The main factor which governs the rate and extent of these reactions under aqueous conditions is pH, which must be lower than 6 for these reactions to occur. Temperature is another critical factor affecting reaction rate. Light is not necessary for acid-catalyzed reaction, but it can induce oxidation reactions to occur. It is important to note that one compound can be converted into many other components. In addition, a single terpene may be formed from many different precursors. The deterioration pathways for limonene and citral, the major aroma components in citrus oil, are examined in this chapter.

Limonene is the monocyclic *p*-menthadiene hydrocarbon which can be hydrated to  $\alpha$ - and  $\beta$ -terpineol under mildly acidic conditions. The former alcohol is the major product because the hydration rate of the exocyclic double bond of limonene is 10 times greater than that of endocyclic double bond (Clark and Chamblee, 1992). Both  $\alpha$ - and  $\beta$ -terpineols are further hydrated to yield secondary products including (*Z*)- and (*E*)-1,8-terpins, and then dehydrated to form 1,8-cineol at high temperature (Clark and Chamblee, 1992). In addition to the above reactions, the double bonds in limonene structure can also rearrange under acidic conditions to form terpinolene, and  $\alpha$ - and  $\gamma$ -terpinenes. 4-Terpineol can also be formed from limonene under strongly acidic conditions.

Rearrangement of citral by acid-catalyzed reactions is more complex. Its deterioration under mildly acidic conditions has been investigated by many researchers (Clark et al., 1977; Kimura et al., 1982; Kimura et al., 1983a, 1983b; Peacock and Kuneman, 1985; Schieberle et al., 1988; Grein et al., 1994; Ueno et al., 2004, 2005; Ueno et al., 2006). In addition, the rearrangement pathway of citral in carbonated beverage model system has been proposed (Lorjaroenphon et al., 2012) as shown in **Figure 2.1**. Citral is the mixture of neral (*cis*-) and geranial (*trans*-) which can rearrange to one another by isomerization reaction to reach an equilibrium ratio of 35:65, respectively (Kimura et al., 1982). This is important since only neral can form carbonium ion “A” by cyclization reaction in aqueous acid solution. Thus, because of the isomerization reaction, both neral and geranial are eventually consumed by the cyclization reaction.

Carbonium ion “A” further deprotonates to form (*Z*)- and (*E*)-isopiperitenols which then rearrange to form (*Z*)- and (*E*)-*p*-mentha-2,8-dien-1-ols, respectively. The carbonium ion can also undergo hydration reaction to form the (*Z*)- and (*E*)-*p*-menth-1-ene-3,8-diols which are extremely unstable (Kimura et al., 1982). These diols can undergo dehydration to form *p*-mentha-1(7),2-dien-8-ol and *p*-mentha-1,5-dien-8-ol, resulting in an equilibration ratio of 45:55 (Kimura et al., 1983a). They can also rearrange to form (*Z*)- and (*E*)-*p*-menth-2-ene-1,8-diols with an equilibration ratio of 42:58 (Lorjaroenphon et al., 2012). These four reaction products can rearrange to one another, but only *p*-mentha-1,5-dien-8-ol and (*Z*)-*p*-menth-2-ene-1,8-diol can form an equilibrium with 2,3-dehydro-1,8-cineol. This latter equilibrium favors the diols over dehydrocineol (Lorjaroenphon et al., 2012).

The compound *p*-mentha-1,5-dien-8-ol also dehydrates to form *p*-cymene, or is converted to *p*-cymen-8-ol by disproportionation and redox reactions, which do not required oxygen

(Kimura et al., 1983a). *p*-Cymen-8-ol is then dehydrated to the more stable  $\alpha$ -*p*-dimethylstyrene which can eventually oxidize to form *p*-methylacetophenone (Schieberle and Grosch, 1988; Schieberle et al., 1988; Ueno et al., 2004). Whether this reaction is actually due to oxidation is uncertain since *p*-methylacetophenone was formed in an oxygen-depleted carbonated beverage model system (Lorjaroenphon et al., 2012).

### **Cinnamon Oil**

Cinnamon (*Cinnamomum zeylanicum*) originates from Southern Asia. Its essential oil can be produced from leaf or stem bark by hydro or steam distillation. The most abundance aroma in leaf oil is eugenol, while stem bark oil contains mainly cinnamaldehyde (Senanayake et al., 1978). Chinese cinnamon or cassia (*Cinnamomum cassia*) oil may be used in cola carbonated beverages as well (Pendergrast, 2000). Cassia oil distilled from leaf and bark contains mainly cinnamaldehyde, with lesser amounts of coumarin, cinnamic acid, and cinnamyl alcohol (Dodge and Sherndal, 1915; ter Heide, 1972; He et al., 2005).

### **Coriander Oil**

Essential oil of coriander (*Coriandrum sativum* L.) used by the beverage industry is obtained from the fruit (sometimes called seed) (Parthasarathy and Zachariah, 2008) by steam or water distillation. It is usually produced from small size fruit (var. *microcarpa*) because of the higher yield compared to large fruit (var. *vulgare*) (Kiralan et al., 2009). The characteristic aroma of coriander oil is pleasant and sweet. The oil contains mainly linalool (about 60-80%), and minor amounts of  $\gamma$ -terpinene, geranyl acetate, and  $\alpha$ -pinene (Kerrola and Kallio, 1993; Gil et al., 2002; Ravi et al., 2007).

## **Neroli Oil**

Neroli oil is obtained from distillation of white blossom of bitter orange tree (*Citrus aurantium* L.) which is a native of China. The floral scent is the top note of this essential oil. Linalool is the most abundance aroma compound found in neroli oil, followed by limonene, nerolidol, and  $\beta$ -ocimene (Toyoda et al., 1993).

## **Nutmeg Oil**

The kernel of the nutmeg (*Myristica fragrans* Houttyn) seed (pericarp) is hydrodistilled to obtain an essential oil which has clove-like, spicy, and sweet characteristic. Two common volatile compounds of nutmeg oil are eugenol and 4-terpineol (Jukić et al., 2006). The percentage of 4-terpineol in the dried seed is higher than those in the fresh seed. Nutmeg oil contains up to 19% of 4-terpineol followed by  $\alpha$ -pinene (14%) (Choo et al., 1999).

## **Vanilla Extract**

Cured vanilla (*Vanilla planifolia*) bean is used to produce vanilla extract which has a typical vanilla and sweet note. The major flavor compounds are vanillin, hydroxybenzaldehyde, and vanillic acid (Hartman et al., 1992). Vanillin is also a dominant compound in nature-identical flavorings, while ethyl vanillin (not present in natural vanilla extract) and/or synthetic vanillin are main components of artificial vanilla (Sostaric et al., 2000; Boyce et al., 2003). Guaiacol, 4-methylguaiacol, acetovanillone, and vanillyl alcohol also possess high odor intensity in vanilla extract, but their concentrations are very low compared to vanillin (Pérez-Silva et al., 2006).

## V. HIGHLIGHTS AND KEY DEVELOPMENTS IN FLAVOR ANALYSIS

Thousands of volatiles have been identified in foods and beverages, but not all of them contribute the aroma of these products. In the beginning, flavor scientists were not often successful in (re-) creating or mimicking a natural flavor system based on the quantification data derived from gas chromatography-mass spectrometry (GC-MS) analysis. Thus, the concept of odor activity value (OAV; also called aroma value, flavor unit, or odor unit) was proposed to estimate the influence of specific odorants to the overall aroma of an analyzed food. OAV is the ratio of the concentration of odorant in food to its odor detection threshold in the food matrix. It is, however, difficult to determine or find in the published literature the odor thresholds of all volatiles present in food. For this reason GC-olfactometry (GCO) was developed and applied to indicate the aroma-active compounds (odorants) in a complex mixture comprised of odorants and non-odorants (volatiles have no or just minimal odors). GCO is the technique which uses the human nose as a GC detector. There are three categories of GCO techniques, including dilution analysis, detection frequency analysis, and perceived intensity analysis (Grosch, 2001). Among these, dilution experiment is used most often to rank the potency of aroma-active compounds on the basis of their odor thresholds in air.

Charm analysis (Acree et al., 1984) and aroma extract dilution analysis (AEDA) (Ullrich and Grosch, 1987) are common GCO dilution techniques. In both cases, the flavor extract is diluted stepwise (serial dilutions of 1:2 or 1:3 are common) and GCO is performed on each dilution to evaluate individual odorants based on their detection thresholds in air. Because both methods are based on the OAV concept, they assume that the response of each aroma compound is linear and all compounds have the same response slopes with decrease in concentration (van Ruth, 2001). After sniffing, the response chromatogram is constructed for Charm analysis in

which peak area is proportional to amount of odorant in the aroma extract. Charm value is a dilution value over the elution time for a specific odorant, while a flavor dilution (FD) factor in AEDA is simpler. An FD factor is the highest dilution for which a panelist can perceive a specific odorant. The dilution technique can also be applied to identify highly volatile compounds by the GCO analysis of decreasing headspace volumes (Holscher and Steinhart, 1992).

The above GCO techniques are valuable screening methods to identify the potent odorants in foods, but they actually only provide relative measures of aroma-activity for compounds in the extract. The effect of food matrix is not taken into account. To help correct this limitation, OAVs are calculated as the next step of the flavor analysis. Any odorant with an OAV greater than one is considered an aroma-active compound. The accuracy of the calculated OAVs is dependent on both accurate quantification and accurate odor threshold determination. Since the aroma compounds in foods differ in terms of their relative abundances and chemical properties, it is nearly impossible to use only a few internal standards for the accurate quantification of all odorants.

In recent years, stable isotope dilution analysis (SIDA) has become the preferred method for accurate and precise quantification of potent odorants in foods. Basically, SIDA is the use of stable isotopically labeled internal standards for quantitative analysis. An isotopologue of each target odorant is prepared by chemical synthesis with site specific labeling with deuterium or  $^{13}\text{C}$ . The labeled volatile is added to the food sample as an internal standard before extraction. It can be assumed that losses of analyte and labeled compound are the same during isolation because the labeled internal standard has the same physical and chemical properties as the target compound. The two isotopologues, which differ in mass, are differentiated by a mass

spectrometry (MS) and the mass ion area ratio of the two compounds is used to determine the abundance of the target analyte in the initial sample.

Besides accurate quantification, it is also necessary to determine accurate odor detection thresholds in the same food matrix for calculation of accurate OAVs. The threshold values in different matrices are not the same and often differ greatly from one another. Interaction between aroma compounds and non-volatile compounds in the food matrix results in different aroma release rates.

The preparation of an aroma reconstitution model is the next step of flavor analysis, and is used to validate the analytical using sensory evaluation methods. This technique is used to overcome the limitation of GCO, which evaluates essentially purified individual compounds as they exit the GC column. In addition, the intensity of aromas with the same OAVs may not be equal, so their contributions to the overall aroma of the product differ.

The model is prepared according to the quantification results by adding the aroma compounds into an odorless food matrix. Sometimes, the matrix is created artificially based on compositional data (Lorjaroenphon et al., 2008). Chetschik et al. (2010) prepared odor-free peanut matrix by removal of the volatile compounds using extraction with different polarity solvents and then freeze-drying the residue. Construction of a model for a solid food is not as simple as for a liquid food due to the lack of homogeneity and also poor distribution of aroma compounds of the case of a solid matrix (Grosch, 2001). It is also important to use high purity aroma compounds for model reconstitution. The odorants which have  $OAVs \geq 1$  are included in the model. However, some aromas for which the  $OAVs < 1$  can be added because their odor qualities may have an additive effect. Descriptive sensory analysis is usually performed to compare the model to the original food product. Sometimes, the initial model, which is based

strictly on the quantification results, is an acceptable match, but sometimes concentration adjustments must be made to create a match.

Omission studies can be conducted once an acceptable aroma reconstitution model is created. The objective of an omission study is to identify which odorants contribute the most to the overall aroma of the food. It can also help to identify if suppression and synergy occurs among the odorants in the aroma mixture. An omission model is prepared by omitting a single odorant or a group of odorants (Czerny et al., 1999). The omission model is subsequently compared to the complete model using sensory evaluation. An odorant is considered to be an important component of the overall aroma of the food, when the omission of that compound causes the omission model to significantly differ from the complete model. There are several sensory techniques which can be used to discriminate the omission model from the complete model, for example duo-trio test (Czerny et al., 1999), triangle test (Christlbauer and Schieberle, 2011), rating scale from extremely different to extremely similar (Dharmawan et al., 2009), descriptive analysis (House and Acree, 2002), and R-index ranking test (Lorjaroenphon et al., 2008).

VI. FIGURE

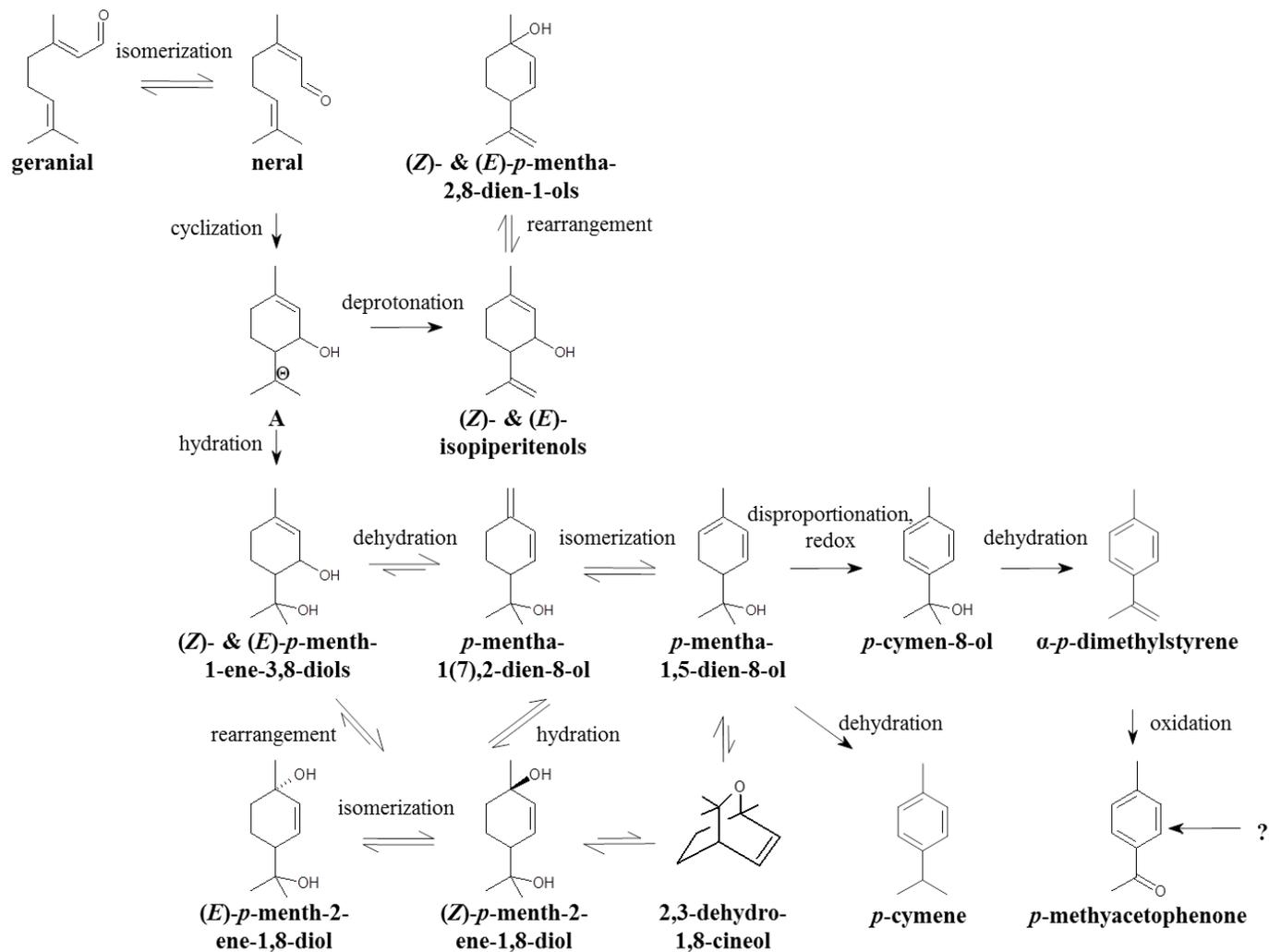


Figure 2.1 Rearrangement pathway of citral in carbonated beverage model system (Lorjaroenphon et al., 2012).

## VII. REFERENCES

- Acree, T.E.; Banard, J.; Cunningham, D.G. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem.* **1984**, *14*, 273-286.
- Boyce, M.C.; Haddad, P.R.; Sostaric, T. Determination of flavour components in natural vanilla extracts and synthetic flavouring by mixed micellar electrokinetic capillary chromatography. *Anal. Chim. Acta* **2003**, *485*, 179-186.
- Chamblee, T.S.; Clark, B.C., Jr.; Brewster, G.B.; Radford, T.; Iacobucci, G.A. Quantitative analysis of the volatile constituents of lemon peel oil. Effects of silica gel chromatography on the composition of its hydrocarbon and oxygenated fractions. *J. Agric. Food Chem.* **1991**, *39*, 162-169.
- Chetschik, I.; Granvogl, M.; Schieberle, P. Quantitation of key peanut aroma compounds in raw peanuts and pan-roasted peanut meal. Aroma reconstitution and comparison with commercial peanut product. *J. Agric. Food Chem.* **2010**, *58*, 11018-11026.
- Choo, L-C.; Wong, S-M.; Liew, K-Y. Essential oil of nutmeg pericarp. *J. Sci. Food Agric.* **1999**, *79*, 1954-1957.
- Christlbauer, M.; Schieberle, P. Evaluation of the key aroma compounds in beef and pork vegetable gravies a la chef by stable isotope dilution assays and aroma recombination experiments. *J. Agric. Food Chem.* **2011**, *59*, 13122-13130.
- Clark, B.C., Jr.; Chamblee, T.S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, 1992; pp 229-285.
- Clark, B.C., Jr.; Powell, C.C.; Radford, T. The acid catalyzed cyclization of citral. *Tetrahedron* **1977**, *33*, 2187-2191.
- Coppella, S.J.; Barton, P. Supercritical carbon dioxide extraction of lemon oil. In *Supercritical Fluids*; Squires, T.G., Paulaitis, M.E., Eds.; ACS Symposium Series 329; American Chemical Society: Washington, DC, 1987; pp 202-212.
- Czerny, M.; Mayer, F.; Grosch, W. Sensory study on the character impact odorants of roasted Arabica coffee. *J. Agric. Food Chem.* **1999**, *47*, 695-699.
- Dharmawan, J.; Kasapis, S.; Sriramula, P.; Lear, M.J.; Curran, P. Evaluation of aroma-active compounds in Pontianak orange peel oil (*Citrus nobilis* Lour. Var. *microcarpa* Hassk.) by gas chromatography-olfactometry, aroma reconstitution, and omission test. *J. Agric. Food Chem.* **2009**, *57*, 239-244.

- Dodge, F.D.; Sherndal, A.E. On constituents of oil of cassia-I. *J. Ind. Eng. Chem.* **1915**, *7*, 1055-1056.
- Elmore, J.S.; Erbahadir, M.A.; Mottram, D.S. Comparison of dynamic headspace concentration on Tenax with solid phase microextraction for the analysis of aroma volatiles. *J. Agric. Food Chem.* **1997**, *45*, 2638-2641.
- Ferhat, M.A.; Meklati, B.Y.; Chemat, F. Comparison of different isolation methods of essential oil from citrus fruits: cold pressing, hydrodistillation and microwave 'dry' distillation. *Flavour Frag. J.* **2007**, *22*, 494-504.
- Fischer, A; Grab, W.; Schieberle, P. Characterisation of the most odour-active compounds in a peel oil extract from Pontianak oranges (*Citrus nobilis* var. *Lour. microcarpa* Hassk.). *Eur. Food Res. Technol.* **2008**, *227*, 735-744.
- Gil, A.; de la Fuente, E.B.; Lenardis, A.E.; Pereira, M.L.; Suarez, S.A.; Bandoni, A.; van Baren, C.; Lira, P.D.L.; Ghersa, C.M. Coriander essential oil composition from two genotypes grown in different environmental conditions. *J. Agric. Food Chem.* **2002**, *50*, 2870-2877.
- Grein, B.; Schmidt, G.; Full, G.; Winterhalter, P.; Schreier, P. 2-Formylmethyl-2-methyl-5-(1-hydroxy-1-methylethyl)-tetrahydrofuran: Major volatile product of the water mediated oxidative decomposition of citral. *Flavour Frag. J.* **1994**, *9*, 93-98.
- Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533-545.
- Hargitt, B. Introduction. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D.P., Ashurst, P.R., Eds.; Blackwell Publishing: Chennai, India, 2006; pp 1-15.
- Hartman, T.G.; Karmas, K.; Chen, J.; Shevade, A.; Deagro, M.; Hwang, H-I. Determination of vanillin, other phenolic compounds, and flavors in vanilla beans. In *Phenolic Compounds in Food and Their Effects on Health I*; Ho, C-T., Lee, C.Y., Huang, M-T., Eds.; ACS Symposium Series 506; American Chemical Society: Washington, DC, 1992; pp 60-76.
- He, Z-D.; Qiao, C-F.; Han, Q-B.; Cheng, C-L.; Xu, H-X.; Jiang, R-W.; But, P.P-H.; Shaw, P-C. Authentication and quantitative analysis on the chemical profile of cassia bark (cortex *cinnamomi*) by high-pressure liquid chromatography. *J. Agric. Food Chem.* **2005**, *53*, 2424-2428.
- Hida, Y.; Matsumoto, M.; Kudo, K.; Imamura, T.; Ikeda, N. Identification of beverages and beverage stains by GC/MS using aroma components as indicators. *Int. J. Legal. Med.* **1998**, *111*, 13-16.

- Högnadóttir, Á.; Rouseff, R.L. Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2003**, *998*, 201-211.
- Holscher, W.; Steinhart, H. Investigation of roasted coffee freshness with an improved headspace technique. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 33-38.
- Horman, J. Syrup preparation and syrup room operations. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D.P., Ashurst, P.R., Eds.; Blackwell Publishing: Chennai, India, 2006; pp 87-111.
- House, K.A.; Acree, T.E. Sensory impact of free fatty acids on the aroma of a model cheddar cheese. *Food Qual. Prefer.* **2002**, *13*, 481-488.
- Jukić, M.; Politeo, O.; Miloš, M. Chemical composition and antioxidant effect of free volatile aglycones from nutmeg (*Myristica fragrans* Houtt.) compared to its essential oil. *Croat. Chem. Acta* **2006**, *79*, 209-214.
- Kappes, S.M.; Schmidt, S.J.; Lee, S-Y. Descriptive analysis of cola and lemon/lime carbonated beverages. *J. Food Sci.* **2006**, *71*, S583-S589.
- Kaul, P.N.; Bhattacharya, A.K.; Rao, B.R.R.; Syamasundar, K.V.; Ramesh, S. Volatile constituents of essential oils isolated from different parts of cinnamon (*Cinnamomum zeylanicum* Blume). *J. Sci. Food Agric.* **2003**, *83*, 53-55.
- Kerrola, K.; Kallio, H. Volatile compounds and odor characteristics of carbon dioxide extracts of coriander (*Coriandrum sativum* L.) fruits. *J. Agric. Food Chem.* **1993**, *41*, 785-790.
- Kimura, K.; Iwata, I.; Nishimura, H. Relationship between acid-catalyzed cyclization of citral and deterioration of lemon flavor. *Agric. Biol. Chem.* **1982**, *46*, 1387-1389.
- Kimura, K.; Nishimura, H.; Iwata, I.; Mizutani, J. Deterioration mechanism of lemon flavor. 2. Formation mechanism of off-odor substances arising from citral. *J. Agric. Food Chem.* **1983a**, *31*, 801-804.
- Kimura, K.; Nishimura, H.; Iwata, I.; Mizutani, J. Identification of acidic substances from deteriorated citral and effects of antioxidants on their formation. *Agric. Biol. Chem.* **1983b**, *47*, 1661-1663.
- Kiralan, M.; Calikoglu, E.; Ipek, A.; Bayrak, A.; Gurbuz, B. Fatty acid and volatile oil composition of different coriander (*Coriandrum sativum*) registered varieties cultivated in Turkey. *Chem. Nat. Compd.* **2009**, *45*, 100-102.
- Lorjaroenphon, Y.; Cadwallader, K.R.; Kim, H.; Lee, S-Y. Evaluation of key odorants in chipotle pepper by quantitative analysis, calculation of odor activity values and omission

- studies. In *Recent Highlights in Flavor Chemistry and Biology*, Proceedings of 8<sup>th</sup> Wartburg Symposium on Flavor Chemistry & Biology, Eisenach, Germany, February 28–March 2, 2007; Hofmann, T., Meyerhof, W., Schieberle, P., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, 2008; pp 191-200.
- Lorjaroenphon, Y.; Hausch, B.J.; Cadwallader, K.R. Formation and stability of 2,3-dehydro-1,8-cineol in a model carbonated beverage system. Proceedings of 13<sup>th</sup> Weurman Flavor Research Symposium, Zaragoza, Spain, September 27-30, 2011; in press.
- Minh Tu, N.T.; Thanh, L.X.; Une, A.; Ukeda, H.; Sawamura, M. Volatile constituents of Vietnamese pummel, orange, tangerine and lime peel oils. *Flavour Frag. J.* **2002**, *17*, 169-174.
- Mitiku, S.B.; Sawamura, M.; Itoh, T.; Ukeda, H. Volatile components of peel cold-pressed oils of two cultivars of sweet orange (*Citrus sinensis* (L.) Osbeck) from Ethiopia. *Flavour Frag. J.* **2000**, *15*, 240-244.
- Njoroge, S.M.; Ukeda, H.; Kusunose, H.; Sawamura, M. Volatile components of Japanese Yuzu and lemon oils. *Flavour Frag. J.* **1994**, *9*, 159-166.
- Parthasarathy, V.A.; Zachariah, T.J. Coriander. In *Chemistry of Spices*; Parthasarathy, V.A., Chempakam, B., Zachariah, Eds.; CAB International: Oxford, U.K., 2008; pp 190-210.
- Peacock, V.E.; Kuneman, D.W. Inhibition of the formation of  $\alpha$ -p-dimethylstyrene and p-cymen-8-ol in a carbonated citral-containing beverage system. *J. Agric. Food Chem.* **1985**, *33*, 330-335.
- Pendergrast, M. *For God, Country and Coca-Cola*; Basic Books: New York, 2000; pp 456-460.
- Pérez-Silva, A.; Odoux, E.; Brat, P.; Ribeyre, F.; Rodriguez-Jimenes, G.; Robles-Olvera, V.; García-Alvarado, M.A.; Günata, Z. GC-MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans. *Food Chem.* **2006**, *99*, 728-735.
- Ranadive, A.S. Vanillin and related flavor compounds in vanilla extracts made from beans of various global origins. *J. Agric. Food Chem.* **1992**, *40*, 1922-1924.
- Ravi, R.; Prakash, M.; Bhat, K.K. Aroma characterization of coriander (*Coriandrum sativum* L.) oil samples. *Eur. Food Res. Technol.* **2007**, *225*, 367-374.
- Sawamura, M.; Tu, N.T.M.; Yu, X.; Xu, B. Volatile constituents of the peel oils of several sweet oranges in China. *J. Essent. Oil Res.* **2005**, *17*, 2-6.
- Schieberle, P.; Ehrmeier, H.; Grosch, W. Aroma compounds resulting from the acid catalysed breakdown of citral. *Z. Lebensm. Unters. Forsch.* **1988**, *187*, 35-39.

- Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
- Senanayake, U.M.; Lee, T.H.; Wills, R.B.H. Volatile constituents of cinnamon (*Cinnamomum zeylanicum*) oils. *J. Agric. Food Chem.* **1978**, *26*, 822-824.
- Simpson, G.I.G.; Jackson, Y.A. Comparison of the chemical composition of East Indian, Jamaican and other West Indian essential oils of *Myristica fragrans* Houtt. *J. Essent. Oil Res.* **2002**, *14*, 6-9.
- Sostaric, T.; Boyce, M.C.; Spickett, E.E. Analysis of the volatile components in vanilla extracts and flavorings by solid-phase microextraction and gas chromatography. *J. Agric. Food Chem.* **2000**, *48*, 5802-5807.
- Staroscik, J.A.; Wilson, A.A. Seasonal and regional variation in the quantitative composition of cold-pressed lemon oil from California and Arizona. *J. Agric. Food Chem.* **1982**, *30*, 835-837.
- Steen, D.P. Carbon dioxide, carbonation and the principles of filling technology. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D.P., Ashurst, P.R., Eds.; Blackwell Publishing: Chennai, India, 2006; pp 112-143.
- Tatlock, W. Water treatment. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D.P., Ashurst, P.R., Eds.; Blackwell Publishing: Chennai, India, 2006; pp 16-47.
- Tchudi, S.N. *Soda Poppery*; Charles Scribner's sons: New York, 1986; pp 3-19, 25-40.
- ter Heide, R. Qualitative analysis of the essential oil of cassia (*Cinnamomum cassia* Blume). *J. Agric. Food Chem.* **1972**, *20*, 747-751.
- Toyoda, T.; Nohara, I.; Sato, T. Headspace analysis of volatile compounds emitted from various citrus blossoms. In *Bioactive Volatile Compounds from Plants*; Teranishi, R., Buttery, R.G., Sugisawa, H., Eds.; ACS Symposium Series 525; American Chemical Society: Washington, DC, 1993; pp 205-219.
- Ueno, T.; Kiyohara, S.; Ho, C-T.; Masuda, H. Potent inhibitory effects of black tea theaflavins on off-odor formation from citral. *J. Agric. Food Chem.* **2006**, *54*, 3055-3061.
- Ueno, T.; Masuda, H.; Ho, C-T. Combined inhibitory effects of catechins with Fe<sup>3+</sup> on the formation of potent off-odorants from citral. In *Phenolic Compounds in Foods and Natural Health Products*; Shahidi, F., Ho, C-H., Eds.; ACS Symposium Series 909; American Chemical Society: Washington, DC, 2005; pp 129-142.
- Ueno, T.; Masuda, H.; Ho, C-T. Formation mechanism of *p*-methylacetophenone from citral via a tert-alkoxy radical intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677-5684.

Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277-282.

van Ruth, S.M. Methods for gas chromatography-olfactometry: a review. *Biomol. Eng.* **2001**, *17*, 121-128.

Wiley, R.C.; Louie, M.K.; Sheu, M-J. Studies on turpentine-like off-odor in cola. *J. Food Sci.* **1984**, *49*, 485-488.

**CHAPTER 3**

**IDENTIFICATION OF POTENT ODORANTS IN**

**COLA-FLAVORED CARBONATED BEVERAGES**

**I. ABSTRACT**

The aroma-active compounds in typical cola-flavored carbonated beverages were characterized using gas chromatography-olfactometry (GCO) and GC-mass spectrometry (GC-MS). The potent odorants in the top three US brands of regular colas were identified by aroma extract dilution analysis (AEDA). Among the numerous odorants identified, eugenol (spicy, clove-like, sweet) and coumarin (sweet, herbaceous) were predominant in all colas. Other predominant odorants in at least one brand included guaiacol (smoky) and linalool (floral, sweet), while 1,8-cineol (minty, eucalyptus-like) was a moderately potent odorant in all colas. Determination of the enantiomeric compositions of selected monoterpenes indicated that, despite its lower relative abundance, (*R*)-(-)-linalool (34.5%), due to its much lower odor detection threshold, was a more potent odorant than the (*S*)-(+)-enantiomer (65.6%). In addition, aroma profiles of colas were determined by sensory descriptive analysis. Lemon-lime and cooling attributes had the highest odor intensities among the eight sensory descriptors identified. The aroma profiles of the three colas were in good agreement with the potent odorants identified by AEDA.

**II. KEYWORDS**

Cola-flavored carbonated beverage; gas chromatography-olfactometry; aroma extract dilution analysis; enantiomer; sensory descriptive analysis

### III. INTRODUCTION

Carbonated beverages are the largest segment of soft drink industry, and cola is the predominant flavor accounting for 54.0% of the global revenues generated by the soft drink and bottled water industry (IBISWorld, 2011). The sales of cola-flavored carbonated beverages in 2010 were about \$14 billion of the soft drink market in the United States (Kaczanowska, 2010a, 2010b). According to product labels and published literature (Tchudi, 1986; Pendergrast, 2000), the uniqueness and complexity of cola is a result of the mixture of natural flavors derived from vanilla extract and essential oils of citrus, cassia or cinnamon, coriander, nutmeg, and neroli.

Despite its popularity, there are only a few studies about the flavor chemistry of cola. Cola aroma has been studied by both sensory (Kappes et al., 2006) and instrumental (Wiley et al., 1984; Elmore et al., 1997; Hida et al., 1998) techniques. Headspace volatiles of de-carbonated colas were evaluated by solid phase microextraction (SPME) and dynamic headspace analysis (Elmore et al., 1997). These researchers reported that terpenes and aldehydes were the major volatiles and those in highest abundance were limonene and a mixture of  $\alpha$ - and  $\gamma$ -terpineol. Limonene along with  $\gamma$ -terpinene and *p*-cymene were identified as the three most abundant headspace volatiles in off-flavored cola (Wiley et al., 1984). The unique volatile, (*Z*)-terpin hydrate, could be used as a chemical marker for criminological purposes to discriminate cola from the other dark color beverages (Hida et al., 1998).

However, the potent odorants of cola have not been reported. Gas chromatography-olfactometry (GCO), which uses the human nose as a detector of GC, was developed and applied to indicate the odorants from the complex mixture of both aroma volatiles and odorless volatiles. There are three general categories of GCO techniques, which include dilution analysis, detection frequency analysis, and perceived intensity analysis (Grosch, 2001). Aroma extract dilution

analysis (AEDA), developed by Grosch and coworkers (Ullrich and Grosch, 1987; Grosch, 1993, 1994), is frequently used. AEDA is a screening method used to rank the relative potency of aroma-active compounds found in foods. It has been used to identify the potent odorants in foods, beverages, and non-foods, including pomegranate juice (Cadwallader et al., 2010) and scented candle (Watcharananun et al., 2009).

In the present study, the aroma-active compounds in typical cola were characterized using GCO and GC-mass spectrometry (GC-MS). The potent odorants in the top three US brands of regular colas were identified by AEDA. These results of this study can provide a foundation for further studies leading to the identification of key aroma components and improvements in the flavor and shelf-life stability of cola-flavored carbonated beverages.

#### **IV. MATERIALS AND METHODS**

##### **Materials**

The top three US brands of regular cola-flavored carbonated beverages (A, B, and C) and the references used in sensory evaluation (**Table 3.1**) were purchased from local markets (Urbana, IL). Odor-free water used in this experiment was prepared by boiling deionized-distilled water to two thirds of its initial volume.

##### **Chemicals**

General reagent or HPLC grade chemicals, such as anhydrous diethyl ether, anhydrous sodium sulfate, dichloromethane, hydrochloric acid, sodium bicarbonate, and sodium chloride, were obtained from Fisher Scientific (Fair Lawn, NJ). Ultra high purity (UHP) nitrogen and UHP helium were purchased from S.J. Smith (Davenport, IA).

**Reference standard compounds.** The standard compounds used to confirm the structures of the aroma compounds listed in **Table 3.2** were supplied by the companies given in parentheses: compounds nos. **1-6, 8b, 9-11, 13, 14, 16, 19-22 (22a and 22b), 24, 25 (25a and 25b), 26-29**, mixture of **30** and **34, 31 (31a and 31b), 32, 37, 38, 41, 43, 45-49, 51, 52, 56**, and **57** (Sigma-Aldrich, St. Louis, MO); **12** (TCI, Portland, OR); **33, 36, and 39** (Bedoukian, Danbury, CT); **40** (Firmenich, Princeton, NJ); mixture of **53** and **54** (Alfa Aesar, Lancashire, United Kingdom); **8a, 22a, 58** (Fluka, Buchs, Switzerland).

### **Synthesis of 2,3-Dehydro-1,8-Cineol**

2,3-Dehydro-1,8-cineol (**7**) used for identification purposes was synthesized in a 2-step procedure modified from the literature (Bugarčić et al., 2004) as described below.

(1)  $\alpha$ -Terpineol (**31**) (1.51 g; 9.8 mmol) was added to a stirred mixture of pyridine (EM Science, Gibbstown, NJ) (0.79 mL; 9.7 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (50 mL). Phenylselenyl chloride (Sigma-Aldrich) (2.05 g; 10.7 mmol) was added, and the mixture stirred for 15 min to complete the reaction. The pale yellow mixture was washed with aqueous 1 M HCl, followed by addition of saturated aqueous NaHCO<sub>3</sub>, and then 50% saturated aqueous NaCl (50 mL each). The CH<sub>2</sub>Cl<sub>2</sub> layer was concentrated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The impurities were eliminated by flash column chromatography on silica gel (Sigma-Aldrich) using CH<sub>2</sub>Cl<sub>2</sub> as mobile phase. The phenylselenyl ether (46% yield) was obtained after removal of the solvent by Vigreux column distillation at 50°C. MS-EI, *m/z* (%): 43 (100), 153 (62), 184 (36), 95 (25), 41 (25), 77 (24), 55 (20), 109 (20), 182 (19), 93 (19).

(2) The phenylselenyl ether (405.3 mg; 1.31 mmol) was dissolved in 6.5 mL of anhydrous tetrahydrofuran (Sigma-Aldrich). The solution was stirred and cooled in an ice-water

bath. Hydrogen peroxide solution (Sigma-Aldrich) (2.24 mL of 3% wt. in water; 2.0 mmol) was added dropwise. The mixture was allowed to reach room temperature, and then stirred for 18 h. Diethyl ether (25 mL) was added, and the mixture was washed twice with water and then twice with 50% saturated aqueous NaCl. The organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the product isolated by vacuum distillation (76% yield, 61.5% purity). MS-EI, *m/z* (%): 109 (100), 79 (29), 43 (27), 124 (19), 94 (17), 39 (9), 77 (9), 41 (9), 110 (8), 93 (7).

### **Isolation of Volatile Compounds**

Water (200 mL) and diethyl ether (150 mL) were added to the extraction chamber of a continuous liquid-liquid extraction (CLLE) apparatus (part no. Z562440; Sigma-Aldrich) equipped with 5°C condenser, 48°C water bath (distillation flask), and magnetic stirrer. One can of cola sample (355 mL) was poured through the cooled condenser to avoid loss of aroma compounds, and rinsed with 50 mL of water. After extraction for 18 h at a condensation rate of 2 drops/s, the solvent layer was concentrated to 50 mL by Vigreux column distillation at 43°C. The volatile compounds were isolated from any non-volatile compounds by solvent assisted flavor evaporation (SAFE) (Engel et al., 1999) according to Watcharananun et al. (2009). The aroma extract was fractionated with aqueous 0.5 M NaHCO<sub>3</sub> (3 × 20 mL) into acidic (aqueous phase) and neutral-basic (organic phase) fractions. The aqueous layer was acidified to pH 2 with aqueous 4 N HCl, and extracted with diethyl ether (3 × 20 mL). Each fraction was washed with saturated aqueous NaCl (2 × 15 mL), concentrated to 10 mL by Vigreux column distillation, and dried over 2 g of anhydrous Na<sub>2</sub>SO<sub>4</sub>. The ether extract was further concentrated to 350 µL, and kept at -70°C until analysis.

## Identification of Aroma-Active Compounds

The aroma extract were analyzed by gas chromatography-olfactometry (GCO) and GC-mass spectrometry (GC-MS).

**GCO.** GCO was conducted using a 6890 GC (Agilent Technologies, Inc., Santa Clara, CA) equipped with a flame ionization detector (FID) and olfactory detection port (DATU Technology Transfer, Geneva, NY). The aroma extract (2  $\mu\text{L}$ ) was injected in the cool on-column mode (+3°C oven tracking mode) to avoid injection bias and reduce the chance for thermal degradation of any labile compounds. Separations were performed on RTX<sup>®</sup>-Wax and RTX<sup>®</sup>-5 SILMS capillary columns (both 15 m  $\times$  0.32 mm i.d.  $\times$  0.5  $\mu\text{m}$  df; Restek, Bellefonte, PA). The column effluent was split 1:5 between the FID (250°C) and olfactory detection port (250°C), respectively. The oven temperature was programmed from 40°C to 225°C at a ramp rate of 10°C/min for the RTX<sup>®</sup>-Wax column or a ramp rate of 6°C/min for the RTX<sup>®</sup>-5 SILMS column, with initial and final hold times of 5 and 20 min, respectively. The flow rate of the helium carrier gas was 2 mL/min.

**GC-MS.** The GC-MS system consisted of a 6890 GC/5973 mass selective detector (Agilent Technologies, Inc.). One microliter of extract was injected into either the Stabilwax<sup>®</sup> (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$  df; Restek) or SAC<sup>TM</sup>-5 (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$  df; Supelco, Bellefonte, PA) capillary columns in the cool on-column mode (+3°C oven tracking mode). The initial oven temperature was 35°C. After 5 min, the oven temperature was increased at 4°C/min to the final temperature (225°C for Stabilwax<sup>®</sup> or 240°C for SAC<sup>TM</sup>-5), and held for 20 min. The flow rate of helium carrier gas was 1 mL/min. The mass spectra were recorded in full scan mode (35-300 a.m.u., scan rate 5.27 scans/s, interface temperature 280°C, and ionization energy 70 eV).

The retention index (RI) of each compound was calculated using the retention time (RT) of that compound compared against the RTs of a series of standard *n*-alkanes (van den Dool and Kratz, 1963). The aroma-active compounds were positively identified based on comparison of their RI values (on two different polarity stationary phases), odor properties, and mass spectra against those of authentic standard compounds to avoid erroneous identifications as described by Molyneux and Schieberle (2007). A compound was considered to be tentatively identified if no authentic standard was available for comparison. In this case mass spectra were compared against those in the NIST2008 mass spectral database, and retention indices were compared to literature values.

### **Aroma Extract Dilution Analysis**

The aroma extract was diluted stepwise (1:3 (v/v)) using diethyl ether as described previous (Watcharananun et al., 2009). Each dilution was performed by GCO on RTX®-Wax column. The flavor dilution (FD) factor for a specific odorant, which is the highest dilution at which panelists can smell that compound, was reported based on averaging the  $\log_3$ FD factors of three panelists (1 female and 2 males; 20-47 years) after rounding off to the nearest whole number.

### **Determination of Enantiomer Composition**

The enantiomeric compositions of aroma-active compounds were investigated by GC-FID using an InertCap<sup>TM</sup> CHIRAMIX column (30 m × 0.32 mm i.d. × 0.25 μm df; GL Sciences Inc., Tokyo, Japan). Two microliters of the aroma extract or standard solution (~1 ppm in dichloromethane) was injected in the hot split (5:1) mode at 250°C. The oven temperature

program started from 100°C for 1 min, then was ramped at 1°C/min to 180°C, and held for 10 min. Helium was used as a carrier gas at a flow rate of 2.5 mL/min. The configuration of each chiral compound was determined based on comparison of its retention time against that of an authentic standard compound. The relative composition of each enantiomer pair in cola A was based on the average from four aroma extracts.

### **Sensory Descriptive Analysis**

The human subject protocol number 11249 was approved by The University of Illinois at Urbana-Champaign Institutional Review Board (IRB). Ten panelists (students and staff; 7 females and 3 males; 22-38 years) were selected based on the participants' abilities to detect and describe differences in aroma characteristics. They were trained for approximately 15 h to evaluate the aroma (by nose) of authentic cola samples consisting of different brands, lots, packages, and colas stored under various conditions. Terminologies with definitions and commercially available references were developed, and are listed in **Table 3.1**. Cola-flavored carbonated beverage (50 mL) was naturally de-carbonated for 30 min in 125-mL Teflon sniff bottle (Nalgene PTFE wash bottle without siphon tube; Nalge Nunc International, Rochester, NY) at room temperature before evaluation. The sample bottles were covered with aluminum foil, and labeled with randomly generated 3-digit codes. The serving orders of test samples were randomized, and the colas were presented one at a time to panelist. In individual booths, panelists evaluated the samples by gently squeezing the bottle and sniffing the expressed air. They were asked to rate the intensity of each attribute on 15-cm line scale with two end anchors from none to strong.

*Statistical analysis.* The mean score of each attribute/term was calculated, and then plotted to establish the aroma profile as spider web plots. Analysis of variance (ANOVA) was performed by SAS<sup>®</sup> program (SAS institute Inc., Cary, NC) to detect significant differences among cola samples. If there was significant difference ( $p$ -value < 0.05), the least significant difference (LSD) was used to check which samples differed.

## V. RESULTS AND DISCUSSION

The top three US brands of regular cola-flavored carbonated beverages were chosen for comprehensive aroma analysis. Continuous liquid-liquid extraction (CLLE) method was used to isolate the aroma compounds from colas because de-carbonation and extraction could be performed at the same time. The aroma extract obtained from each cola by CLLE had a characteristic cola-like odor. This confirmed that the extract contained the typical aroma-active compounds of the colas.

### Volatile Components

Total ion chromatograms were similar for all three of the cola-flavored carbonated beverage samples (**Figure A.1**). Monoterpenes and sesquiterpenes were the major compounds in colas. Among these, the most abundant volatile components were limonene (**8**),  $\alpha$ -terpineol (**31**), and  $\gamma$ -terpinene (**10**). However, not all volatile compounds found in foods significantly contribute to food aroma (Mistry et al., 1997). In the past, flavor scientists were not successful in creating aroma reconstitution models by only considering the most abundance volatile components. For this reason, gas chromatography-olfactometry (GCO) was developed and

applied to indicate the aroma-active compounds (odorants) in a complex mixture comprised of odorants and non-odorants (volatiles having no or just minimal odors).

### **Aroma-Active Components**

A combined total of 58 odorants were detected in the colas (**Table 3.2**), of which two were unidentified. Most of the odorants found in colas originate from the flavoring ingredients including vanilla extract and essential oils of citrus, cassia or cinnamon, coriander, nutmeg, and neroli (Tchudi, 1986; Pendergrast, 2000). However, some may have been derived from non-volatile components, for example, furfural (**20**) which was reported as degradation product of ascorbic acid in citrus juice (Lee and Nagy, 1996) or acid hydrolysis product of *d*-fructose (Shaw et al., 1967). Moreover, some of these compounds can be derived from other odorants such as formation of  $\alpha$ -terpineol (**31**) from limonene (**8**) due to acid-catalyzed reaction (Clark and Chamblee, 1992).

The concentration and odor detection threshold are two parameters that are important in estimating the aroma impact of a volatile compound in a food. The odor activity value (OAV) concept has been used for decades to estimate to what degree a volatile component might influence the overall aroma of a food. OAV is the ratio of the concentration of an aroma compound in the food to its odor detection threshold in the food matrix. The accuracy of an OAV depends on both accurate quantification and on accurate odor threshold determination. It is difficult to measure odor detection thresholds of all odorants found in colas especially for any unknown compounds. For this reason aroma extract dilution analysis (AEDA) was used to indicate the potent odorants in the present study.

## Potent Odorants

The AEDA technique was used to rank the odorants by their relative potency on the basis of OAVs in air. The flavor dilution (FD) factors of all odorants found in the colas are shown in **Table 3.2**, and the  $\log_3$  FD chromatograms are shown in **Figure B.1**. Eugenol (**51**) (spicy, clove-like, sweet) and coumarin (**56**) (sweet, herbaceous) were predominant odorants in all colas. These odorants have been reported in cinnamon and cassia (Chinese cinnamon) oils (Dodge and Sherndal, 1915; Senanayake et al., 1978; He et al., 2005). Eugenol is also a common volatile compound of nutmeg oil (Jukié et al., 2006).

Guaiacol (**41**) (smoky note) was a potent odorant in colas A and B, while linalool (**22**) (floral, sweet) had high odor potency in cola C. Linalool also had relatively high potency in cola A. This odorant is reported as one of the most aroma-active compounds contributing to the overall flavor of lemon oil (Schieberle and Grosch, 1988) and orange peel oil (Fischer et al., 2008). It is also a major component of the essential oils of coriander (Kerrola and Kallio, 1993; Gil et al., 2002; Ravi et al., 2007) and neroli (Toyoda et al., 1993). In addition, linalool is reported as a dominant alcohol in orange oil (Sawamura et al., 2005), and it was perceived in highest aroma intensity after octanal and wine lactone (Högnadóttir and Rouseff, 2003).

The compound 1,8-cineol (**9**) (minty, eucalyptus-like) was a moderately potent odorant in all colas. However, the most abundant volatiles, including limonene,  $\alpha$ -terpineol, and  $\gamma$ -terpinene, had low odor potencies in the colas. The FD factors of highly potent odorants found in citrus oil, such as neral (**30**) and geranial (**34**), were also low in all colas because of their possible degradation by acid-catalyzed reactions.

It is well known that citral (a mixture of neral and geranial), which is a major component of lemon oil, is extremely unstable under acidic conditions. The half-life of citral in carbonated

soft drinks is about six days (Peacock and Kuneman, 1985). Acid-catalyzed reactions of citral under various conditions can result in the formation of variety of products (Clark et al., 1977). Oxidation of citral can occur under certain conditions (Kimura et al., 1983), but this is unlikely to occur to any significant extent in the case of carbonated beverages due to the very low level of oxygen in these products.

The degradation mechanism for citral has already been proposed (Clark et al., 1977; Kimura et al., 1982; Kimura et al., 1983; Peacock and Kuneman, 1985; Lorjaroenphon et al., 2012). Neral (*cis*-isomer) and geranial (*trans*-isomer) can rearrange by isomerization reactions to one another in an acid environment. Then, *p*-menthadien-8-ols can be converted directly from neral by the series of acid-catalyzed reactions starting from cyclization. These intermediates are not stable under acidic conditions. They are degraded by several reactions including disproportionation, redox, and dehydration reactions to form  $\alpha$ ,*p*-dimethylstyrene (**17**) and *p*-cymene (**11**) which are more stable. The compound 2,3-dehydro-1,8-cineol (**7**) is also formed as a result of the deterioration of citral by acid-catalyzed reactions. This compound is very unstable in acid conditions, but it is found at low levels in colas due to the steady-state reached among various precursor compounds via reversible acid-catalyzed reactions (Lorjaroenphon et al., 2012).

Among the three brands, the aroma profile of cola A was the most complex. Additional moderately potent odorants in cola A included methyleugenol (**46**) (hay-like, dried grass), 4-terpineol (**25**) (earthy, soapy, woody), (*E*)-cinnamaldehyde (**48**) (cinnamon-like, sweet), (*Z*)- and (*E*)-isoeugenols (**53** and **54**) (clove-like, sweet), and an unknown with an RI of 2399 on the RTX<sup>®</sup>-wax column (**55**) (clove-like, sweet). It is interesting to note that 4-terpineol, which is a major volatile in nutmeg oil (Choo et al., 1999), may be a degradation product of some terpenes

via acid-catalyzed reactions (Clark and Chamblee, 1992). It was also reported to be a potent odorant in a stored emulsion of lemon oil in an aqueous citric acid solution (Schieberle and Grosch, 1988).

$\beta$ -Damascenone (**40**) (apple sauce, sweet) was another moderately potent odorant in cola C. Vanillin (**58**), however, was not detected by GCO in this beverage. This finding suggests that vanilla extract may be used at only a low concentration in cola C, possibly as a cost reduction strategy. The low level of vanillin is also supported by low potency of guaiacol (**41**) in the cola since both compounds are important aroma components of vanilla extract (Pérez-Silva et al., 2006). Furthermore, it was indicated that the main component influencing the sweet note of cola C was coumarin, not vanillin. Tonka bean extract which contains coumarin is reportedly used as a vanilla substitute. Coumarin mixed with vanillin is also used in cream soda because it is not highly volatile and its aroma intensity is three times higher than vanillin (Abernethy, 1969). Although coumarin is a toxic compound (Lake, 1999), it occurs naturally in cinnamon oil (Senanayake et al., 1978) and vanilla extract (Boyce et al., 2003).

### **Enantiomeric Composition**

Cola A was chosen to investigate the enantiomeric composition of selected aroma-active compounds since some of them had high FD factors. Furthermore, different enantiomers may have different odor detection thresholds and odor characteristics. The chemical structures and enantiomeric distributions of chiral compounds identified in cola A are shown in **Figure 3.1**. *d*-Limonene or the (*R*)-(+)-isomer (**8b**) was present in greatest abundance (97.2%). This is to be expected because it is well known that *d*-limonene is commonly present in citrus oils. Linalool found in natural flavor is usually present as a mixture of (*R*)-(-)-isomer (**22a**) and (*S*)-(+)-isomer

(**22b**) at different ratios. The enantiomeric composition of linalool in cola A was 34.5% of the (*R*)-(-)- and 65.5% of the (*S*)-(+)-enantiomer. (*R*)-(-)-Linalool is found in neroli which is the white blossom of bitter orange tree (*Citrus aurantium* L.), while (*S*)-(+)-linalool is the predominant enantiomer in coriander (Opdyke, 1975). Although the amount of (*S*)-(+)-linalool in cola was almost two fold higher than (*R*)-(-)-linalool, the odor threshold in water of the latter enantiomer is about nine times lower than that of former stereoisomer (0.8 and 7.4 ppb, respectively; Padrayuttawat et al., 1997). Thus, (*R*)-(-)-linalool should make a greater aroma contribution than (*S*)-(+)-linalool in cola-flavored carbonated beverage.

In addition, the enantiomeric compositions of the 4-terpineols (51.0% of the (*S*)-(+)-isomer (**25a**) and 49.0% of the (*R*)-(-)-isomer (**25b**)) and  $\alpha$ -terpineols (55.1% of the (*S*)-(-)-enantiomer (**31a**) and 44.9% of the (*R*)-(+)-enantiomer (**31b**)) in cola occurred as approximately racemic mixtures. The enantiomeric pairs of 2,3-dehydro-1,8-cineol (**7**), isborneol (**29**) and borneol (**32**) were, however, not determined on the chiral GC capillary column because of their low concentrations in cola extracts.

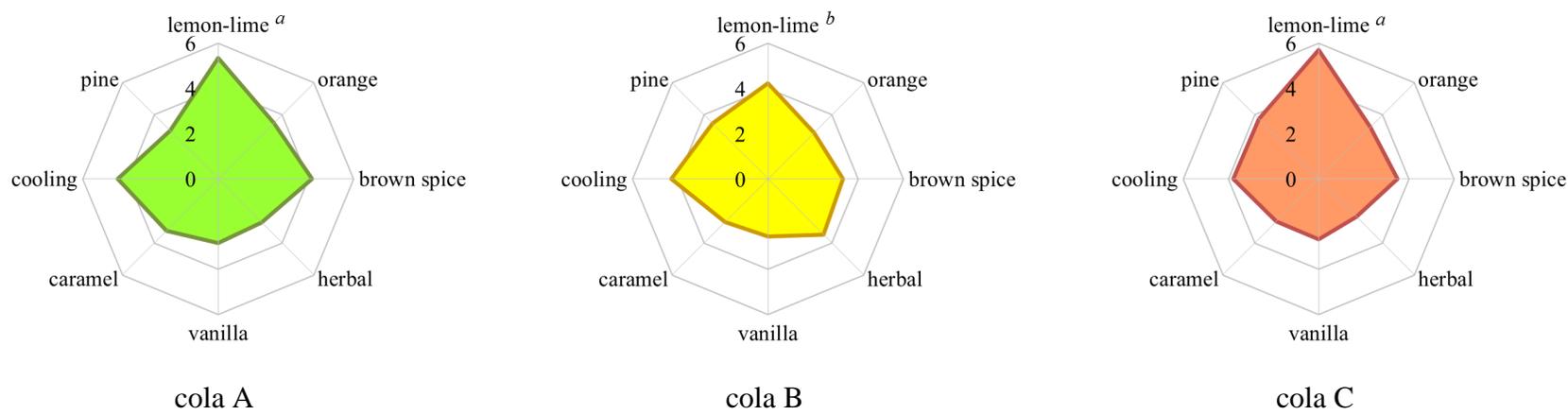
## Sensory Analysis

Aroma (by nose) of de-carbonated colas was described by eight attributes including lemon-lime, orange, brown spice, herbal, vanilla, caramel, cooling, and pine (**Table 3.1**). Citrus, caramel and vanilla have been reported as aromas and aromatics (aroma-by-mouth) of regular and diet colas by descriptive analysis (Kappes et al., 2006). In the present study, all colas (A, B, and C) had similar aroma profiles, except the lemon-lime intensity in cola B was significantly lower than in colas A and C ( $p$ -value < 0.05) (**Figure 3.2** and **Table D.1**). Lemon-lime and cooling attributes were dominant attributes in all colas. Brown spice, herbal, and pine attributes were

moderate intensities in all colas. The results of sensory analysis agreed well with potent odorants identified by AEDA (**Table 3.2**). The low intensity of the lemon-lime note in cola B was probably due to the low potency of linalool (**22**) in that product, while the cooling note of cola could be associated with a higher level of 1,8-cineol (**9**). The high potency aroma compounds were also responsible for the other aroma attributes, such as brown spice aroma from eugenol (**51**) or vanilla note from coumarin (**56**). Additionally, the moderately spicy note of cola A compared to the other colas was possibly the result of the impact of methyleugenol (**46**) and isoeugenols (**53** and **54**).

While AEDA is the useful screening technique to rank the aromas in order of potency, the method is, however, based on the odor-activity of the aroma compound in air. The effect of the food matrix on cola odor was not taken into account in the present study. These potent odorants identified in colas were further analyzed by OAVs to correct for the limitations of AEDA, and additional sensory studies on an aroma reconstitution and omission models are presented in **Chapter 4** of this dissertation.





**Figure 3.2** Aroma (by nose) profiles of cola-flavored carbonated beverages from sensory descriptive analysis. Attributes followed by different letters are significantly different ( $p$ -value < 0.05). Average intensities correspond to those in **Table D.1** ( $n = 10$ ; 7 females and 3 males; 22-38 years).

**Table 3.1** Attributes, definitions, and references used for sensory descriptive analysis of cola-flavored carbonated beverages.

attribute	definition	reference		
		product (Mfg.)	preparation <sup>a</sup>	intensity <sup>b</sup>
lemon-lime	aromatic reminiscent of lemon or lime	Sprite <sup>®</sup> (lemon-lime soda) (Coca-Cola Co., Atlanta, GA)	50 mL	7
orange	aromatic reminiscent of orange	Sunkist <sup>®</sup> (orange soda) (Sunkist Growers, Inc., Sherman Oaks, CA)	50 mL	9
brown spice	aromatic associate with allspice, cinnamon, clove, mace, nutmeg (spicy)	whole cloves (McCormick & Co., Inc., Hunt Valley, MD)	0.2 g	10
herbal	aromatic associate with dried herbs, hay or grass	dried long grass (cut into 2 cm.)	3 g	9
vanilla	vanilla-like aroma	vanillin (compound no. <b>58</b> ; Fluka, Buchs, Switzerland)	10 mL (50 ppm in water)	8
caramel	sweet aromatic	Werther's Original <sup>®</sup> (chewy caramels) (made in Germany for Storck USA L.P., Chicago, IL)	1 piece (~6 g)	6
cooling	cooling aroma, but not identical to mint	Halls <sup>®</sup> (Mentho-Lyptus) (made in Canada; dist: Cadbury Adams USA LLC, Parsippany, NJ)	10 mL (3.5 mg/mL water)	7
pine	aromatic reminiscent of pine needles	Pine-Sol <sup>®</sup> (original) (Fabricado Para the Clorox Co., Oakland, CA)	10 mL (100 ppm in water)	7

<sup>a</sup>Prepared in 125-mL Teflon sniff bottle. <sup>b</sup>Relative intensity on 15-cm line with two anchor scales from none to strong.

**Table 3.2** Aroma-active compounds in three commercial brands of regular cola-flavored carbonated beverages.

no. <sup>a</sup>	RI <sup>b</sup>		F <sup>d</sup>	compound	odor <sup>e</sup>	FD factor <sup>f</sup>			identification <sup>g</sup>
	wax	RTX <sup>®</sup> -5				A	B	C	
<b>1</b>	1019	939	NB	$\alpha$ -pinene	camphorous, cool	27	< 3	3	RI, O, MS, S
<b>2</b>	1073	941 <sup>c</sup>	NB	camphene	camphorous	3	-	3	RI, O, MS, S
<b>3</b>	1108	-	NB	$\beta$ -pinene	fresh	9	9	9	O, S
<b>4</b>	1154	1013	NB	$\delta$ -3-carene	citrus, minty	9	27	3	RI, O, MS, S
<b>5</b>	1175	993	NB	$\beta$ -myrcene	rubbery, terpene	3	3	3	RI, O, MS, S
<b>6</b>	1186	1025	NB	$\alpha$ -terpinene	rubbery, pine	3	9	9	RI, O, MS, S
<b>7</b>	1208	986 <sup>c</sup>	NB	2,3-dehydro-1,8-cineol	minty	< 3	-	< 3	RI, O, MS, S
<b>8</b>	1221	1048	NB	limonene	orange, terpene	< 3	-	3	RI, O, MS, S
<b>9</b>	1225	1044	NB	1,8-cineol	minty, eucalyptus	81	81	81	RI, O, MS, S
<b>10</b>	1252	1072	NB	$\gamma$ -terpinene	terpene	< 3	-	< 3	RI, O, MS, S
<b>11</b>	1271	1024 <sup>c</sup>	NB	<i>p</i> -cymene	herbal, pine	< 3	9	3	RI, O, MS, S
<b>12</b>	1297	1089	NB	$\alpha$ -terpinolene	pine, plastic	-	3	9	RI, O, MS, S
<b>13</b>	1307	1005	NB	octanal	orange, citrus	9	9	9	RI, O, MS, S
<b>14</b>	1340	-	NB	6-methyl-5-hepten-2-one	mushroom	3	-	< 3	O, MS, S
<b>15</b>	1380	-	NB	unknown	fruity	27	9	9	O
<b>16</b>	1416	1111	NB	nonanal	orange, green, sweet, fresh	9	3	9	RI, O, MS, S

**Table 3.2** (continued)

no. <sup>a</sup>	RI <sup>b</sup>		F <sup>d</sup>	compound	odor <sup>e</sup>	FD factor <sup>f</sup>			identification <sup>g</sup>
	wax	RTX <sup>®</sup> -5				A	B	C	
<b>17</b>	1434	-	NB	$\alpha$ - <i>p</i> -dimethylstyrene	orange, fresh	3	3	3	O, MS
<b>18</b>	1445	1070 <sup>c</sup>	NB	linalool oxide	floral	3	3	-	RI, O, MS
<b>19</b>	1463	< 700 <sup>c</sup>	A	acetic acid	vinegar	< 3	< 3	< 3	RI, O, MS, S
<b>20</b>	1475	829 <sup>c</sup>	NB	furfural	sweet	9	-	-	RI, O, MS, S
<b>21</b>	1517	1205 <sup>c</sup>	NB	decanal	orange, fresh, green	< 3	9	3	RI, O, MS, S
<b>22</b>	1555	1102	NB	linalool	floral, sweet	243	9	729	RI, O, MS, S
<b>23</b>	1592	1130	NB	1-terpineol	soapy	-	-	< 3	RI, O, MS
<b>24</b>	1602	1415 <sup>c</sup>	NB	$\beta$ -caryophyllene	earthy, woody	27	3	9	RI, O, MS, S
<b>25</b>	1623	1191	NB	4-terpineol	earthy, soapy, woody	81	3	9	RI, O, MS, S
<b>26</b>	1637	807 <sup>c</sup>	A	butanoic acid	cheesy, buttery	< 3	< 3	< 3	RI, O, MS, S
<b>27</b>	1669	860 <sup>c</sup>	A	2-furanmethanol	popcorn, sweet	< 3	-	-	RI, O, MS, S
<b>28</b>	1681	888	A	3-methylbutanoic acid	cheesy, stinky feet	< 3	< 3	< 3	RI, O, S
<b>29</b>	1688	1175	NB	isoborneol	earthy, camphorous	27	9	9	RI, O, MS, S
<b>30</b>	1699	-	NB	neral	soapy, lemon	9	9	3	O, MS, S
<b>31</b>	1713	1206	NB	$\alpha$ -terpineol	phenolic, pine	3	< 3	9	RI, O, MS, S
<b>32</b>	1723	1184	NB	borneol	earthy, camphorous, phenolic	3	< 3	< 3	RI, O, MS, S

**Table 3.2** (continued)

no. <sup>a</sup>	RI <sup>b</sup>			compound	odor <sup>e</sup>	FD factor <sup>f</sup>			identification <sup>g</sup>
	wax	RTX <sup>®</sup> -5	F <sup>d</sup>			A	B	C	
<b>33</b>	1735	1531	NB	(+)-valencene	camphorous, earthy	27	3	-	RI, O, MS, S
<b>34</b>	1755	-	NB	geranial	sweet, terpene	< 3	-	-	O, MS, S
<b>35</b>	1759	-	NB	( <i>E</i> )-piperitol	saffron, straw, hay-like, stale	9	< 3	-	O, MS
<b>36</b>	1799	1238	NB	nerol	floral, sweet	27	3	3	RI, O, MS, S
<b>37</b>	1841	-	NB	2-phenethyl acetate	floral	< 3	-	< 3	O, MS, S
<b>38</b>	1843	1325	NB	( <i>E,E</i> )-2,4-decadienal	fatty, fried, oily	3	9	3	RI, O, MS, S
<b>39</b>	1854	1259 <sup>c</sup>	NB	geraniol	cleaner, lemon pledge	-	-	< 3	RI, O, MS, S
<b>40</b>	1857	-	NB	β-damascenone	apple sauce, sweet	27	< 3	81	O, S
<b>41</b>	1878	-	NB	guaiacol	smoky	729	243	27	O, MS, S
<b>42</b>	1893	1284 <sup>c</sup>	NB	safrol	burnt sugar, spice	3	3	< 3	RI, O, MS
<b>43</b>	1935	1120	NB	2-phenylethanol	rose, sweet	27	27	3	RI, O, MS, S
<b>44</b>	1970	1238 <sup>c</sup>	NB	2-methoxybenzaldehyde	herbaceous, lactone, sweet, vanilla, grape	27	-	9	RI, O, MS
<b>45</b>	1981	1114	A	maltol	sweet	< 3	-	< 3	RI, O, MS, S
<b>46</b>	2004	1403 <sup>c</sup>	NB	methyleugenol	hay-like, dried grass	243	27	3	RI, O, MS, S
<b>47</b>	2053	1064	NB	2,5-dimethyl-4-hydroxy-3( <i>2H</i> )- furanone (Furaneol <sup>TM</sup> )	burnt sugar, sweet	9	9	< 3	RI, O, S

**Table 3.2** (continued)

no. <sup>a</sup>	RI <sup>b</sup>			compound	odor <sup>e</sup>	FD factor <sup>f</sup>			identification <sup>g</sup>
	wax	RTX <sup>®</sup> -5	F <sup>d</sup>			A	B	C	
<b>48</b>	2091	1283	NB	( <i>E</i> )-cinnamaldehyde	cinnamon, sweet	81	3	3	RI, O, MS, S
<b>49</b>	2100	1093 <sup>c</sup>	NB	<i>p</i> -cresol	dung, animal stable	9	27	27	RI, O, MS, S
<b>50</b>	2147	1442 <sup>c</sup>	NB	cinnamylacetate	cloves, cinnamon, sweet	9	3	3	RI, O, MS
<b>51</b>	2195	1361	NB	eugenol	spicy, cloves, sweet	729	243	729	RI, O, MS, S
<b>52</b>	2220	1130	A	3-hydroxy-4,5-dimethyl-2(5 <i>H</i> )-furanone (sotolon)	burnt sugar, curry, spicy	9	3	9	RI, O, S
<b>53</b>	2299	1411	NB	( <i>Z</i> )-isoeugenol	cloves, sweet	81	27	9	RI, O, S
<b>54</b>	2373	1456	NB	( <i>E</i> )-isoeugenol	sweet, cloves	81	< 3	3	RI, O, S
<b>55</b>	2399	-	NB	unknown	cloves, sweet	81	< 3	3	O
<b>56</b>	2507	1451	NB	coumarin	sweet, herbaceous	729	243	729	RI, O, MS, S
<b>57</b>	2583	1263 <sup>c</sup>	A	phenylacetic acid	rosy, sweet, plastic	9	3	< 3	RI, O, MS, S
<b>58</b>	2596	1404	A	vanillin	vanilla	27	9	-	RI, O, MS, S

<sup>a</sup>Numbers correspond to those in **Figure 3.1** and **Chapter 4**. <sup>b</sup>Retention indices determined by GCO on two different stationary phases (RTX<sup>®</sup>-Wax and RTX<sup>®</sup>-5 SILMS). <sup>c</sup>RI determined by GC-MS. <sup>d</sup>Fraction: acidic (A); neutral-basic (NB). <sup>e</sup>Odor quality determined by GCO. <sup>f</sup>Flavor dilution factors were determined on RTX<sup>®</sup>-Wax column, and were derived from average log<sub>3</sub>FD factors (n = 3; 1 female and 2 males; 20-47 years) after rounding off to the nearest whole number.

<sup>g</sup>Identification criteria: retention index (RI); odor quality (O); mass spectra (MS); reference standard compound (S).

## VII. REFERENCES

- Abernethy, J.L. The historical and current interest in coumarin. *J. Chem. Educ.* **1969**, *46*, 561-568.
- Boyce, M.C.; Haddad, P.R.; Sostaric, T. Determination of flavour components in natural vanilla extracts and synthetic flavouring by mixed micellar electrokinetic capillary chromatography. *Anal. Chim. Acta* **2003**, *485*, 179-186.
- Bugarčić, Z.M.; Dunkić, J.D.; Mojsilović, B.M. A simple, convenient and expeditious approach to cineol. *Heteroatom Chem.* **2004**, *15*, 468-470.
- Cadwallader, K.R.; Tamamoto, L.C.; Sajuti, S.C. Aroma components of fresh and stored pomegranate (*Punica granatum* L.). In *Flavors in Noncarbonated Beverages*; Da Costa, N.C., Cannon, R.J., Eds.; ACS Symposium Series 1036; American Chemical Society: Washington, DC, 2010; pp 93-101.
- Choo, L-C.; Wong, S-M.; Liew, K-Y. Essential oil of nutmeg pericarp. *J. Sci. Food Agric.* **1999**, *79*, 1954-1957.
- Clark, B.C., Jr.; Chamblee, T.S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, 1992; pp 229-285.
- Clark, B.C., Jr.; Powell, C.C.; Radford, T. The acid catalyzed cyclization of citral. *Tetrahedron* **1977**, *33*, 2187-2191.
- Dodge, F.D.; Sherndal, A.E. On constituents of oil of cassia-I. *J. Ind. Eng. Chem.* **1915**, *7*, 1055-1056.
- Elmore, J.S.; Erbahadir, M.A.; Mottram, D.S. Comparison of dynamic headspace concentration on Tenax with solid phase microextraction for the analysis of aroma volatiles. *J. Agric. Food Chem.* **1997**, *45*, 2638-2641.
- Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237-241.
- Fischer, A; Grab, W.; Schieberle, P. Characterisation of the most odour-active compounds in a peel oil extract from Pontianak oranges (*Citrus nobilis* var. *Lour. microcarpa* Hassk.). *Eur. Food Res. Technol.* **2008**, *227*, 735-744.
- Gil, A.; de la Fuente, E.B.; Lenardis, A.E.; Pereira, M.L.; Suarez, S.A.; Bandoni, A.; van Baren, C.; Lira, P.D.L.; Ghersa, C.M. Coriander essential oil composition from two genotypes grown in different environmental conditions. *J. Agric. Food Chem.* **2002**, *50*, 2870-2877.

- Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68-73.
- Grosch, W. Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Frag. J.* **1994**, *9*, 147-158.
- Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533-545.
- He, Z-D.; Qiao, C-F.; Han, Q-B.; Cheng, C-L.; Xu, H-X.; Jiang, R-W.; But, P.P-H.; Shaw, P-C. Authentication and quantitative analysis on the chemical profile of cassia bark (cortex cinnamomi) by high-pressure liquid chromatography. *J. Agric. Food Chem.* **2005**, *53*, 2424-2428.
- Hida, Y.; Matsumoto, M.; Kudo, K.; Imamura, T.; Ikeda, N. Identification of beverages and beverage stains by GC/MS using aroma components as indicators. *Int. J. Legal. Med.* **1998**, *111*, 13-16.
- Högnadóttir, Á.; Rouseff, R.L. Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2003**, *998*, 201-211.
- IBISWorld. Global Soft Drink & Bottled Water Manufacturing. *Global Industry Report C1124-GL* [Online], November 9, 2011, p 14. IBISWorld.  
<http://clients.ibisworld.com/globalindustry/default.aspx?indid=420> (accessed January 12, 2012).
- Jukić, M.; Politeo, O.; Miloš, M. Chemical composition and antioxidant effect of free volatile aglycones from nutmeg (*Myristica fragrans* Houtt.) compared to its essential oil. *Croat. Chem. Acta* **2006**, *79*, 209-214.
- Kaczanowska, A. Carbonated soft drink production in the US. *IBISWorld Industry Report 31211a* [Online], December, 2010b, p 12. IBISWorld.  
<http://www.ibisworld.com/industryus/default.aspx?indid=285> (accessed December 9, 2010).
- Kaczanowska, A. Soft drink production in the US. *IBISWorld Industry Report 31211* [Online], August, 2010a, pp 13-14. IBISWorld.  
<http://www.ibisworld.com/industryus/default.aspx?indid=284> (accessed December 9, 2010).
- Kappes, S.M.; Schmidt, S.J.; Lee, S-Y. Descriptive analysis of cola and lemon/lime carbonated beverages. *J. Food Sci.* **2006**, *71*, S583-S589.

- Kerrola, K.; Kallio, H. Volatile compounds and odor characteristics of carbon dioxide extracts of coriander (*Coriandrum sativum* L.) fruits. *J. Agric. Food Chem.* **1993**, *41*, 785-790.
- Kimura, K.; Iwata, I.; Nishimura, H. Relationship between acid-catalyzed cyclization of citral and deterioration of lemon flavor. *Agric. Biol. Chem.* **1982**, *46*, 1387-1389.
- Kimura, K.; Nishimura, H.; Iwata, I.; Mizutani, J. Deterioration mechanism of lemon flavor. 2. Formation mechanism of off-odor substances arising from citral. *J. Agric. Food Chem.* **1983**, *31*, 801-804.
- Lake, B.G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. *Food Chem. Toxicol.* **1999**, *37*, 423-453.
- Lee, H.S.; Nagy, S. Chemical degradative indicators to monitor the quality of processed and stored citrus products. In *Chemical Markers for Processed and Stored Foods*; Lee, T-C., Kim, H-J., Eds.; ACS Symposium Series 631; American Chemical Society: Washington, DC, 1996; pp 86-106.
- Lorjaroenphon, Y.; Hausch, B.J.; Cadwallader, K.R. Formation and stability of 2,3-dehydro-1,8-cineol in a model carbonated beverage system. Proceedings of 13<sup>th</sup> Weurman Flavor Research Symposium, Zaragoza, Spain, September 27-30, 2011; in press.
- Mistry, B.S.; Reineccius, T.; Olson, L.K. Gas chromatography-olfactometry for the determination of key odorants in foods. In *Techniques for Analyzing Food Aroma*; Marsili, R., Ed.; Marcel Dekker: New York, 1997; pp 265-292.
- Molyneux, R.J.; Schieberle, P. Compound identification: A journal of agricultural and food chemistry perspective. *J. Agric. Food Chem.* **2007**, *55*, 4625-4629.
- Opdyke, D.L.J. Linalool. *Food Cosmet. Toxicol.* **1975**, *13*, 827-832.
- Padrayuttawat, A.; Yoshizawa, T.; Tamura, H.; Tokunaga, T. Optical isomers and odor thresholds of volatile constituents in *Citrus sudachi*. *Food Sci. Technol. Int. Tokyo* **1997**, *3*, 402-408.
- Peacock, V.E.; Kuneman, D.W. Inhibition of the formation of  $\alpha$ -*p*-dimethylstyrene and *p*-cymen-8-ol in a carbonated citral-containing beverage system. *J. Agric. Food Chem.* **1985**, *33*, 330-335.
- Pendergrast, M. *For God, Country and Coca-Cola*; Basic Books: New York, 2000; pp 456-460.
- Pérez-Silva, A.; Odoux, E.; Brat, P.; Ribeyre, F.; Rodriguez-Jimenes, G.; Robles-Olvera, V.; García-Alvarado, M.A.; Günata, Z. GC-MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans. *Food Chem.* **2006**, *99*, 728-735.

- Ravi, R.; Prakash, M.; Bhat, K.K. Aroma characterization of coriander (*Coriandrum sativum* L.) oil samples. *Eur. Food Res. Technol.* **2007**, *225*, 367-374.
- Sawamura, M.; Tu, N.T.M.; Yu, X.; Xu, B. Volatile constituents of the peel oils of several sweet oranges in China. *J. Essent. Oil Res.* **2005**, *17*, 2-6.
- Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
- Senanayake, U.M.; Lee, T.H.; Wills, R.B.H. Volatile constituents of cinnamon (*Cinnamomum zeylanicum*) oils. *J. Agric. Food Chem.* **1978**, *26*, 822-824.
- Shaw, P.E.; Tatum, J.H.; Berry, R.E. Acid-catalyzed degradation of *d*-fructose. *Carbohydr. Res.* **1967**, *5*, 266-273.
- Tchudi, S.N. *Soda Poppery*; Charles Scribner's sons: New York, 1986; pp 32-34.
- Toyoda, T.; Nohara, I.; Sato, T. Headspace analysis of volatile compounds emitted from various citrus blossoms. In *Bioactive Volatile Compounds from Plants*; Teranishi, R., Buttery, R.G., Sugisawa, H., Eds.; ACS Symposium Series 525; American Chemical Society: Washington, DC, 1993; pp 205-219.
- Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277-282.
- van den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463-471.
- Watcharananun, W.; Cadwallader, K.R.; Huangrak, K.; Kim, H.; Lorjaroenphon, Y. Identification of predominant odorants in Thai desserts flavored by smoking with "Tian Op", a traditional Thai scented candle. *J. Agric. Food Chem.* **2009**, *57*, 996-1005.
- Wiley, R.C.; Louie, M.K.; Sheu, M-J. Studies on turpentine-like off-odor in cola. *J. Food Sci.* **1984**, *49*, 485-488.

**CHAPTER 4**

**CHARACTERIZATION OF POTENT ODORANTS IN A COLA-FLAVORED  
CARBONATED BEVERAGE BY QUANTITATIVE ANALYSIS AND SENSORY  
STUDIES OF AROMA RECONSTITUTION AND OMISSION MODELS**

**I. ABSTRACT**

Thirty aroma-active components of cola-flavored carbonated beverage were accurately quantified by stable isotope dilution analysis (SIDA), and the odor activity values (OAVs) of selected compounds were calculated based on their odor detection thresholds in water. Based on their highest overall OAVs, 1,8-cineol, (*R*)-(-)-linalool, and octanal made the greatest contribution to the overall aroma of cola, followed by nonanal, (*S*)-(+)-linalool, decanal, and (*R*)-(+)-limonene. Aroma recombination studies were conducted to evaluate potential interactions among selected key aroma compounds in a simulated beverage matrix. The cola aroma reconstitution model was constructed based on quantification data by adding twenty high purity standards to an aqueous sucrose-phosphoric acid solution. The aroma profile by sensory descriptive analysis indicated that herbal and pine notes of the cola model were significantly higher than those of the de-carbonated authentic cola. Headspace-solid phase microextraction (HS-SPME) and additional sensory analyses were used to adjust the model to better match authentic cola. The aroma profile of the re-balanced model did not differ from that of the authentic cola, and was used as a complete model for the omission study. The omission models were constructed by omitting groups of odorants instead of single odorants. Results of sensory aroma comparisons (R-index ranking tests) indicated that only one omission model, comprised of methyleugenol, (*E*)-cinnamaldehyde, eugenol, and (*Z*)- and (*E*)-isoeugenols, differed from the

complete model. The omission models constructed by the omission of the individual components of this group did not differ from the complete model. These results indicate that a balance of numerous odorants is responsible for the characteristic aroma of cola-flavored carbonated beverages.

## II. KEYWORDS

Cola-flavored carbonated beverage; odor activity value; stable isotope dilution analysis; aroma reconstitution model; omission study

## III. INTRODUCTION

Cola is the predominant flavor among the various types of commercial carbonated beverages. Despite its popularity, there is limited information about the flavor chemistry of cola. In our previous study, 58 odorants from three commercial brands of regular cola were identified by gas chromatography-olfactometry (GCO) and aroma extract dilution analysis (AEDA) (**Chapter 3**). The compounds eugenol (spicy, clove-like, sweet) and coumarin (sweet, herbaceous) were the most potent odorants in all colas. Other potent odorants in some colas included guaiacol (smoky) and linalool (floral, sweet). (*R*)-(-)-Linalool despite being the less abundant enantiomer was regarded as a more potent odorant than the (*S*)-(+)-enantiomer because of its much lower odor detection threshold. The analytical data also correlated well with aroma profiles generated by sensory descriptive analysis.

The application of AEDA is a useful method for ranking the relative potency of odorants, but the interactions between the odorants and the food matrix is not taken into account. Calculation of odor activity values (OAVs) is proposed as a next step of flavor analysis to correct

for this limitation (Grosch, 2001). OAV of specific odorant is the ratio of its concentration in the food to its odor detection threshold in the food matrix. This concept aims to estimate the contribution of specific odorants to the overall aroma of the food. Any odorant with an OAV greater than one is considered as an aroma-active compound. The accuracy of the calculated OAVs is dependent on both accurate quantification and availability or determination of accurate odor thresholds. Odor thresholds should be evaluated in the same food matrix for accuracy. Meanwhile, stable isotope dilution analysis (SIDA) can be applied for accurate quantification of specific odorants since the physical and chemical properties of isotopic labeled internal standard compound are similar to those of target analytes (Mistry et al., 1997).

It is, however, necessary to verify the analytical data by aroma recombination studies due to both known and unidentified limitations of GCO and the OAV concept (Grosch, 2001). Interactions among odorants are not taken into account when using GCO since the odorants are separated before evaluation at the sniffing port. Furthermore, the contribution of compounds with the same OAVs may not be equal because of different odor intensities. In addition, odorants with  $OAV < 1$  may be sensed due to an additive effect.

An aroma reconstitute model can be prepared by adding high purity standard odorants into identical food matrix which is obtained from analytical data (Lorjaroenphon et al., 2008) or to an odorless food base. Then the aroma attributes of the model are compared by sensory evaluation to the original product. Occasionally it is necessary to make adjustments to the model composition in order for it to match the original product. The matched model can also be used as a complete model for omission studies.

The objective of an omission study is to identify the character-impact odorants which must be present in order for the product to have its typical aroma. An omission model is

prepared by omitting a single odorant or a group of aroma components (Czerny et al., 1999). The omission model is further compared to the complete model to investigate which odorants contribute the most to overall aroma of the food. Furthermore, suppression and synergistic effects can be identified by omission study as well (Lorjaroenphon et al., 2008).

The objectives of the present study were to: 1) quantify the potent odorants by SIDA, 2) identify key potent odorants by calculation of OAVs, and 3) investigate character-impact odorants by sensory evaluation of aroma reconstitution and omission models. Knowledge of key odorants which contribute to typical flavor of cola could be used as quality indicator compounds to develop products with superior flavors and to investigate the flavor changes which occur during storage.

#### **IV. MATERIALS AND METHODS**

##### **Materials**

Samples of a single commercial brand of regular cola-flavored carbonated beverage and the references for sensory descriptive analysis were purchased from local markets (Urbana, IL).

##### **Chemicals**

General reagent or HPLC grade chemicals including anhydrous diethyl ether, anhydrous sodium sulfate, dichloromethane, hydrochloric acid, methanol, phosphoric acid, sodium bicarbonate, sodium chloride, and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ). Sucrose was supplied by Sigma-Aldrich (St. Louis, MO). Liquid nitrogen, ultra high purity (UHP) nitrogen, and UHP helium were obtained from S.J. Smith (Davenport, IA).

**Standard compounds.** The standard compounds listed in **Tables 4.1 – 4.3** were supplied by the companies given in parentheses: compound nos. **8b, 9, 13, 16, 19, 21, 22 (22a and 22b), 25 (25a and 25b), 26, 28, 29 (29a and 29b)**, mixture of **30 and 34, 31 (31a and 31b), 32a, 32b, 37, 41, 43, 46, 48, 49, 51, 56, and 57** (Sigma-Aldrich); **36 and 39** (Bedoukian, Danbury, CT); **40** (Firmenich, Princeton, NJ); mixture of **53 and 54** (Alfa Aesar, Lancashire, United Kingdom); **58** (Fluka, Buchs, Switzerland). All compounds for reconstitution study were purified by vacuum distillation and/or flash column chromatography to obtain the high purity standards before using. The enantiomeric distribution of compound nos. **8b, 22, 25, 29, 31, 32a, and 32b** were determined using the method described in **Chapter 3**.

**Isotope standard compounds.** The following isotopically labeled standards were obtained from the commercial sources listed in parentheses: [ $^2\text{H}_3$ ]-acetic acid (**I-19**), [ $^2\text{H}_7$ ]-butanoic acid (**I-26**), 2-methoxy- $^2\text{H}_3$ -phenol ( $^2\text{H}_3$ -guaiacol) (**I-41**), and [ $^2\text{H}_3$ ]-*p*-cresol (**I-49**) (CDN, Quebec, Canada); [ $^{13}\text{C}_2$ ]-phenylacetic acid (**I-57**) (Isotec, Miamisburg, OH).

## Syntheses

The following compounds were synthesized according to the procedures reported in the literature given in parentheses: [ $^2\text{H}_2$ ]-limonene (**I-8**) (Chen et al., 1998); [ $^2\text{H}_2$ ]-linalool (**I-22**) (Steinhaus et al., 2003); [ $^2\text{H}_2$ ]-3-methylbutanoic acid (**I-28**) (Steinhaus and Schieberle, 2005); [ $^2\text{H}_4$ ]- $\beta$ -damascenone (**I-40**) (Kotseridis et al., 1998); [ $^{13}\text{C}_2$ ]-2-phenylethanol (**I-43**) (Schuh and Schieberle, 2006); [ $^2\text{H}_3$ ]-vanillin (**I-58**) (Schneider and Rolando, 1992).

[ $^2\text{H}_4$ ]-*Octanal* (**I-13**). This isotopically labeled standard was prepared in two steps adapted from the literature (Lin et al., 1999; Corey and Suggs, 1975).

(1) Chlorotri(triphenylphosphine)rhodium(I) (Wilkinson's catalyst; Sigma-Aldrich) (15 wt % of the alkynol; 0.15 g), 3-octyn-1-ol (1.0 g; 7.9 mmol), and 5 mL of [ $^2H$ ]-methanol (Sigma-Aldrich) were placed in a pressure reactor equipped with stir bar and rubber septum. The reactor was flushed for 5 min with deuterium gas (40 psi; UHP grade 99.995%; isotopic enrichment 99.7%; Matheson Tri-Gas, Parsippany, NJ) using a needle, which was placed below the solution. The spent catalyst was removed by centrifugation after the reaction was complete. [ $^2H_4$ ]-Octan-1-ol was obtained after purification by vacuum distillation (0.79 g, 5.9 mmol, 75% yield). MS-EI,  $m/z$  (%): 43 (100), 57 (97), 42 (88), 58 (86), 44 (83), 56 (76), 41 (73), 72 (65), 71 (65), 59 (51).

(2) Labeled alcohol from previous step (0.41 g; 3 mmol) in 2 mL of  $CH_2Cl_2$  was added to 10 mL suspension of pyridinium chlorochromate (Sigma-Aldrich) (1.1 g; 0.005 mol) in  $CH_2Cl_2$ . The mixture was stirred for 1.5 h at room temperature, and 20 mL of diethyl ether was added. The supernatant was decanted, and the residue was extracted with diethyl ether ( $3 \times 10$  mL) until the black gum became granular in consistency. The combined organic phase was filtered through 10 g bed of Florisil<sup>®</sup> (Sigma-Aldrich). The extract was purified by vacuum distillation before removal of solvent to obtain the standard compound (0.18 g, 27% yield, 92.2% purity). MS-EI,  $m/z$  (%): 45 (100), 59 (71), 44 (68), 43 (63), 58 (62), 57 (48), 42 (45), 41 (44), 46 (42), 56 (40).

[ $^2H_4$ ]-Nonanal (**I-16**). This labeled aldehyde was prepared in the same manner as described above. [ $^2H_4$ ]-Nonan-1-ol (4.7 mmol, 66% yield) (MS-EI,  $m/z$  (%): 43 (100), 57 (81), 56 (78), 41 (74), 42 (68), 58 (64), 44 (63), 71 (51), 72 (49), 70 (43)) was oxidized to form the

aldehyde (2.0 mmol, 66% yield, 93.6% purity). MS-EI,  $m/z$  (%): 45 (100), 59 (97), 43 (75), 57 (66), 58 (65), 41 (63), 56 (60), 44 (58), 60 (46), 42 (46).

$[^2H_4]$ -Decanal (**I-21**). The preparation of this isotope compound was the same as previously stated.  $[^2H_4]$ -Decan-1-ol (5.0 mmol, 77% yield) (MS-EI,  $m/z$  (%): 43 (100), 57 (97), 42 (88), 58 (86), 44 (83), 56 (76), 41 (73), 72 (65), 71 (65), 59 (51)) was oxidized to aldehyde (1.9 mmol, 63% yield, 95.2% purity). MS-EI,  $m/z$  (%): 43 (100), 59 (88), 45 (86), 41 (67), 57 (59), 70 (54), 56 (53), 44 (52), 58 (49), 42 (48).

$[^2H_7]$ -4-Terpineol (**I-25**). The synthesis was performed in two steps following the procedure reported in the literature for  $[^2H_3]$ -*p*-mentha-1,8-dien-4-ol (Masanetz and Grosch, 1998b) with some modifications as detailed below.

(1) Anhydrous ammonia gas (Matheson TriGas, Basking Ridge, NJ) was condensed to 100 mL at  $-78^\circ\text{C}$  (keeping the temperature between  $-80$  and  $-75^\circ\text{C}$ ), and diethyl ether (50 mL) was added. 4-Methylanisole (Sigma-Aldrich) (4.89 g; 40 mmol) was dissolved, and stirred for 15 min. Small pieces of lithium ribbon (Sigma-Aldrich) (0.38 g; 55 mmol) were slowly added into the solution (dark color appeared), and stirred for 90 min. Then, ethanol (Acros Organics, Fair Lawn, NJ) (50 mL) was dropped into the solution within 1 h. The mixture was allowed to reach room temperature (dark color disappeared), and the reaction was stopped by adding ice-cold water (100 mL). The aqueous layer was extracted with diethyl ether ( $3 \times 50$  mL). The pooled ether extract was washed with water ( $2 \times 50$  mL), and hydrolyzed with saturated aqueous oxalic acid (Sigma-Aldrich) (125 mL) for 14 h. The aqueous layer was further extracted with diethyl ether ( $2 \times 50$  mL), and combined organic layer was washed with aqueous 0.5 M  $\text{NaHCO}_3$

(2 × 50 mL) and then water (2 × 100 mL). The solution was concentrated by Vigreux column distillation at 43°C, and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The target compound was purified by flash column chromatography on silica gel (Sigma-Aldrich) using 5% followed by 20% diethyl ether in pentane as mobile phases. 4-Methylcyclohex-3-enone was collected after removal of solvent (0.33 g, 7% yield, 93.9% purity). MS-EI, *m/z* (%): 67 (100), 68 (69), 110 (46), 53 (30), 41 (20), 40 (15), 42 (13), 51 (12), 50 (11), 52 (8).

(2) Magnesium turnings (Sigma-Aldrich) (0.11 g; 4.4 mmol) in diethyl ether (10 mL) were placed in three-necked flask equipped with condenser at 10°C under N<sub>2</sub> purge line, and cooled in an ice-water bath. A few drops of [<sup>2</sup>H<sub>7</sub>]-2-bromopropane (Isotec) was added into the stirred mixture. A crystal of iodine (EM Science, Gibbstown, NJ) was also added to initiate the reaction. [<sup>2</sup>H<sub>7</sub>]-2-Bromopropane was further added drop-by-drop until the magnesium turnings disappeared (~2.28 g). After stirring for 1 h, 4-methylcyclohex-3-enone (0.33 g; 3 mmol) in diethyl ether (5 mL) was slowly added through an anhydrous Na<sub>2</sub>SO<sub>4</sub> column. The solution was allowed to reach room temperature under reflux, then capped and stirred overnight. Ice-cold water (50 mL) was added to stop the reaction, and the mixture was adjusted to pH 3 with aqueous 2 M HCl. The aqueous layer was immediately extracted with diethyl ether (3 × 50 mL). The combined extracts were washed with water (2 × 50 mL), and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before removal of the solvent. The residue was fractionated on a silica gel column using 30% then 50% diethyl ether in pentane, and purified by vacuum distillation. After checking the mass spectrum, the solution was washed with aqueous 0.5 M NaOH to remove an unexpected *p*-cresol by product. The solution was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the solvent was evaporated to obtain [<sup>2</sup>H<sub>7</sub>]-4-terpineol (23.6 mg, 5% yield, 70.2% purity). MS-EI, *m/z* (%): 93 (100), 75 (93), 111 (80), 78 (70), 50 (43), 55 (33), 69 (27), 46 (24), 41 (22), 43 (22).

$[^2\text{H}_3]$ -Isoborneol (**I-29**) and  $[^2\text{H}_3]$ -borneol (**I-32**). The six-step procedure used to synthesize these compounds was adapted from the literature (Havens and Meloan, 1995; Furniss et al., 1989; Corey and Suggs, 1975) as detailed below.

(1) (1*S*)-(+)-Ketopinic acid (Sigma-Aldrich) (1.06 g; 5.82 mmol), methanol (21 g; 640 mmol), and concentrated sulfuric acid (0.26 g) were placed in a 50-mL screw cap test tube and sealed with Teflon lined cap. The solution was heated at 65°C overnight, and then allowed to reach room temperature. The mixture was neutralized with aqueous 1 M NaHCO<sub>3</sub>, excess methanol was evaporated, and the product extracted with diethyl ether. The solvent layer was washed with saturated aqueous NaCl before drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Methyl ketopinate was obtained after evaporation of solvent (54.1% yield). MS-EI, *m/z* (%): 95 (100), 41 (80), 67 (79), 39 (76), 109 (55), 93 (49), 153 (40), 108 (39), 79 (36), 55 (34).

(2) Lithium aluminum deuteride (Sigma-Aldrich) (0.210 g; 4.99 mmol) was prepared in pre-dried 50-mL centrifuge tube equipped with stirrer and N<sub>2</sub> purge line. Diethyl ether (20 mL) was added, and the mixture was cooled in an ice-water bath under a N<sub>2</sub> stream. Methyl ketopinate (0.618 g; 3.15 mmol) in 5 mL of diethyl ether was added drop by drop within 2 min into the stirred mixture. The tube was capped, and warmed to room temperature. After stirring the mixture for 2 h, the tube was placed in ice-water bath. Deuterium oxide (Sigma-Aldrich) (2 mL) and then water (10 mL) were poured to deactivate the excess LiAlD<sub>4</sub>. The mixture was acidified to pH < 2 by aqueous 2 M H<sub>2</sub>SO<sub>4</sub>. The aqueous layer was extracted with diethyl ether (2 × 20 mL) followed by washing with saturated NaCl and drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The crude  $[^2\text{H}_{3-5}]$ -1-(hydroxymethyl)-7,7-dimethylbicyclo[2.2.1]heptan-2-ol (0.62 g) obtained after evaporating off solvent was kept for the next step. MS-EI (isomer 1), *m/z* (%): 110 (100), 95 (56), 109 (41), 41 (37), 94 (34), 43 (29), 69 (22), 42 (22), 80 (20), 111 (20). MS-EI (isomer 2),

$m/z$  (%): 110 (100), 95 (83), 41 (42), 109 (40), 94 (29), 43 (28), 69 (23), 42 (23), 55 (20), 67 (20).

(3) The product from previous step (0.62 g) was combined with 5 mL of dichloromethane in a 50-mL test tube cooled in an ice-water bath equipped with stirrer. Pyridine (EM Science, Gibbstown, NJ) (0.51 mL; 6.30 mmol) and then *p*-toluenesulfonyl chloride (Fulka, St. Louis, MO) (0.905 g; 4.7 mmol) were slowly added into the vigorously stirred mixture. After 48 h, pyridine (0.26 mL; 3.21 mmol) and *p*-toluenesulfonyl chloride (0.449 g; 2.36 mmol) were added and the solution stirred for 24 h. Diethyl ether and then water was added to stop the reaction. The ether phase was washed with aqueous 2 M HCl and then 5% aqueous NaHCO<sub>3</sub>. The product was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before removal of solvent to yield crude [<sup>2</sup>H<sub>3-4</sub>]-(*2*-hydroxy-7,7-dimethylbicyclo[2.2.1]heptan-1-yl)methyl-4-methylbenzenesulfonate (1.1 g). MS-EI,  $m/z$  (%): 114 (100), 43 (76), 96 (74), 41 (62), 67 (51), 68 (45), 95 (42), 39 (41), 42 (40), 83 (38).

(4) In a pre-dried round bottom flask equipped with N<sub>2</sub> purge line, LiAlD<sub>4</sub> (0.15 g; 3.6 mmol) was dissolved in 20 mL of diethyl ether. The solution of intermediate from prior step prepared in diethyl ether (5 mL) was added dropwise. The mixture was stirred over several days, and small portions of LiAlD<sub>4</sub> were added. The reaction was stopped and extracted in the same manner as described in step 3. A crude mixture of [<sup>2</sup>H<sub>4-5</sub>]-isoborneol and [<sup>2</sup>H<sub>4-5</sub>]-borneol was formed (0.51 g). MS-EI ([<sup>2</sup>H<sub>4-5</sub>]-isoborneol),  $m/z$  (%): 98 (100), 95 (73), 41 (44), 43 (36), 97 (35), 96 (30), 39 (24), 113 (24), 42 (23), 69 (23). MS-EI ([<sup>2</sup>H<sub>4-5</sub>]-borneol),  $m/z$  (%): 98 (100), 95 (66), 41 (34), 113 (26), 99 (25), 43 (19), 69 (18), 42 (17), 44 (15), 39 (14).

(5) This step followed the procedure of Corey and Suggs (1975). The suspension of pyridinium chlorochromate (Sigma-Aldrich) (1.12 g; 5.19 mmol) and anhydrous CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was prepared in 50-mL vial equipped with stirrer. Crude [<sup>2</sup>H<sub>4-5</sub>]-isoborneol and [<sup>2</sup>H<sub>4-5</sub>]-

borneol (0.51 g) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added one portion to the suspension. The mixture was stirred for 1.5, and diethyl ether (20 mL) was added. The supernatant was decanted, and the residue was extracted with diethyl ether (3 × 10 mL) until the black gum became granular. The pooled extract was filtered through a bed of Florisil<sup>®</sup> (Sigma-Aldrich) (10 g). The solvent was evaporated by Vigreux column distillation at 47°C to yield crude [<sup>2</sup>H<sub>3</sub>]-camphor (0.32 g). Further purification was done by flash column chromatography using 5% diethyl ether in pentane as eluting solvent. MS-EI, *m/z* (%): 84 (100), 98 (83), 41 (66), 95 (58), 111 (53), 39 (46), 86 (44), 44 (42), 72 (40), 155 (34).

(6) Borane-tetrahydrofuran complex (Sigma-Aldrich) (0.839 mL; 0.839 mmol) and tetrahydrofuran (2 mL) were prepared in 25-mL centrifuge tube equipped with ice-water bath, stirrer and N<sub>2</sub> purge line. [<sup>2</sup>H<sub>3</sub>]-Camphor (105.7 mg; 0.68 mmol) in 2 mL of tetrahydrofuran was added drop by drop within 2 min. The tube was removed from the ice-water bath, and sealed with periodic venting. The mixture was stirred until the reaction was complete. The reaction was stopped, and extracted in the same manner as described in step 3 (95.3% total purity) (48.3% purity of **I-29** and 47.0% purity of **I-32**). MS-EI (**I-29**), *m/z* (%): 98 (100), 95 (71), 113 (33), 96 (29), 41 (18), 139 (18), 124 (18), 43 (16), 82 (14), 55 (13). MS-EI (**I-32**), *m/z* (%): 98 (100), 95 (62), 113 (36), 41 (14), 96 (12), 43 (11), 142 (10), 139 (9), 55 (9), 69 (8).

[<sup>2</sup>H<sub>3</sub>]-*α*-Terpineol (**I-31**). This compound was prepared in two steps adapted from the literature (Inukai and Kojima, 1965, 1966).

(1) Isoprene (Sigma-Aldrich) (0.68g; 10 mmol) and 3-buten-2-one (Sigma-Aldrich) (0.71 g; 10 mmol) were dissolved in 20 mL of CH<sub>2</sub>Cl<sub>2</sub>. The solution was cooled to -78°C with stirring before adding aluminum chloride (Sigma-Aldrich) (0.13 g; 1 mmol). The mixture was

allowed to warm, and stirred at room temperature for 3 h. The solution was washed with 10% (w/v) aqueous Na<sub>2</sub>SO<sub>4</sub> (50 mL). The solvent phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before removal of non-volatiles by vacuum distillation. The solvent was evaporated to obtain 1-acetyl-4-methylcyclohex-3-ene (73% yield). MS-EI, *m/z* (%): 43 (100), 95 (76), 67 (76), 39 (59), 138 (58), 41 (43), 79 (42), 123 (37), 55 (33), 77 (29).

(2) [<sup>2</sup>H<sub>3</sub>]-Methylolithium (Sigma-Aldrich) (20 mL of 0.5M in diethyl ether; 10 mmol) was prepared in three-neck round bottom flask equipped with stirrer, N<sub>2</sub> purge line, and vent needle. The solution was cooled in ice-water bath (~0°C) before adding 1-acetyl-4-methylcyclohex-3-ene (1.02g; 7.3 mmol) in 5 mL of diethyl ether through syringe needle. The mixture was stirred at ~0°C for 2 h. Saturated aqueous NH<sub>4</sub>Cl (20 mL) was added drop by drop before adding 20 mL of water. The mixture was extracted with diethyl ether (3 × 20 mL), and the organic layer was washed with saturated aqueous NaCl (2 × 20 mL). The extract was concentrated by Vigreux column distillation at 43°C before purification on silica gel column using 20% ether in pentane as eluting solvent. The yield after removal of solvent was 81% (94.5% purity). MS-EI, *m/z* (%): 62 (100), 93 (63), 139 (55), 81 (39), 124 (31), 121 (25), 92 (23), 46 (18), 43 (18), 67 (17).

[<sup>2</sup>H<sub>3</sub>]-1,8-cineol (**I-9**). This isotope was prepared in two steps by following the procedure reported previously for cineol synthesis (Bugarčić et al., 2004; Nicolaou et al., 1980). The details are as follows.

(1) [<sup>2</sup>H<sub>3</sub>]- $\alpha$ -Terpineol (**I-31**) (0.16 g; 1 mmol) was phenylselenoetherified by pyridine (EM Science) (0.08 g; 1 mmol) and phenylselenenyl chloride (Sigma-Aldrich) (0.21 g; 1.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL). The reaction was completed in 15 min, and yielded a pale yellow solution. The mixture was washed with aqueous 1 M HCl (5 mL), saturated aqueous NaHCO<sub>3</sub>

(5 mL), and 50% saturated aqueous NaCl (5 mL), respectively. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the labeled target compound was isolated from diphenyl diselenide on a silica gel column using CH<sub>2</sub>Cl<sub>2</sub> as the mobile phase. Phenyl selenoether of [<sup>2</sup>H<sub>3</sub>]-1,8-cineol was obtained after removal of solvent. MS-EI, *m/z* (%): 43 (100), 156 (70), 184 (33), 77 (27), 95 (24), 46 (23), 112 (21), 182 (18), 41 (18), 138 (17).

(2) Final product from the above step was mixed with toluene (2.5 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and then added to a high pressure tube under N<sub>2</sub> gas. The mixture of tributyltin hydride (Sigma-Aldrich) (0.58 g; 2 mmol) and 2,2'-azobisisobutyro-nitrile (Aldrich, Steinheim, Germany) (1 mL of 0.02 M toluene solution; 0.02 mmol) was added (pale yellow color disappeared), and stirred under N<sub>2</sub> gas for 15 min. Then, the tube was sealed with a Teflon cap, and kept in GC oven at 110°C for 1 h. Saturated aqueous NaCl (5 mL) was added to stop the reduction reaction. The mixture was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and solvent was evaporated. The residue was passed through silica gel column using 5% diethyl ether in pentane, and the solvent was removed to obtain [<sup>2</sup>H<sub>3</sub>]-1,8-cineol (18% yield, 96.1% purity). MS-EI, *m/z* (%): 43 (100), 81 (58), 111 (57), 87 (54), 72 (52), 157 (43), 71 (37), 96 (36), 95 (31), 46 (29).

*[<sup>2</sup>H<sub>6</sub>]-nerol (I-36) and [<sup>2</sup>H<sub>6</sub>]-geraniol (I-39).* The two isotopes were prepared by adapting the procedure of Durst and Leete (1971).

(1) Vinylmagnesium bromide (1.0 M in THF; Sigma-Aldrich) (100 mL; 0.10 mol) was placed in a three neck flask equipped with stir bar and N<sub>2</sub> purge line. The flask was chilled at 0°C in an ice-water bath. [<sup>2</sup>H<sub>6</sub>]-Acetone (Sigma-Aldrich) (7.6 g; 0.12 mol) in 10 mL of diethyl ether was slowly added to the stirred solution. The color of the solution immediately turned from dark brown to yellow. The mixture was stirred at 0°C for 4 h, and was neutralized with

aqueous 0.1 M HCl. The pH of mixture was adjusted to be slightly acidic with saturated aqueous  $\text{NH}_4\text{Cl}$  solution before extraction with diethyl ether ( $4 \times 30$  mL). The pooled organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated to 20 mL using Vigreux column distillation at  $70^\circ\text{C}$  to yield crude [ $^2\text{H}_6$ ]-2-methyl-3-buten-2-ol. The solution was dried over anhydrous  $\text{Na}_2\text{SO}_4$  again to remove any water before using in the next step. MS-EI,  $m/z$  (%): 46 (100), 74 (74), 65 (32), 44 (19), 43 (13), 42 (11), 75 (9), 55 (7), 41 (7), 40 (6).

(2) In a pre-dried 250-mL round bottom flask equipped with a mechanical stirrer and a  $\text{N}_2$  purge line, phosphorus tribromide (Sigma-Aldrich) (32.0 g; 0.12 mol) was dissolved in 75 mL of pentane. The mixture was stirred and cooled to  $-10^\circ\text{C}$  in an ice-salt bath. The product from the first step was combined with 3.75 g of pyridine (EM Science), and then slowly added into the mixture and stirred at  $-10^\circ\text{C}$ . The solution was brownish color in the upper pentane layer and gummy on the bottom layer. After 2 h, 10 mL of water was added to stop the reaction. The pentane layer was separated, and the aqueous phase (middle layer) was extracted with pentane ( $3 \times 30$  mL). The combined pentane extract was washed with 5% aqueous  $\text{Na}_2\text{CO}_3$  ( $2 \times 25$  mL), 5% aqueous HCl ( $1 \times 25$  mL), and then saturated aqueous NaCl ( $2 \times 25$  mL). The extract was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to 20 mL before purification by vacuum distillation. The distillate was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and the solvent was evaporated to obtain [ $^2\text{H}_6$ ]-3,3-dimethylallylbromide (52% purity) (3.9 g; 25.1 mmol). MS-EI,  $m/z$  (%): 75 (100), 44 (66), 45 (47), 43 (26), 42 (20), 46 (14), 41 (13), 40 (10), 56 (9), 55 (7).

(3) Sodium ethoxide solution (21 wt. %; Sigma-Aldrich) (25 mL; 66.3 mmol sodium), ethyl acetoacetate (Sigma-Aldrich) (11.7 g; 90 mmol), and 15 mL of absolute ethanol (Acros Organics) were placed in round bottom flask equipped with glass boiling beads. The product from previous step in 1 mL of ethanol was added dropwise to the mixture at room temperature,

and refluxed for 12 h. Aqueous 50% w/v aqueous KOH solution (7 mL) was added, and the mixture was further refluxed for 2.5 h. The reaction mixture was cooled, acidified with aqueous 25% w/v aqueous H<sub>2</sub>SO<sub>4</sub> solution to pH 2-4, and diluted with 100 mL water. The aqueous layer was extracted with diethyl ether (2 × 50 mL). The pooled extract was washed with saturated aqueous NaCl (3 × 25 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated to 20 mL by Vigreux column distillation. The extract was subjected to vacuum distillation to remove any non-volatiles before evaporation of solvent. The synthesis product was further purified by flash column chromatography using 30% diethyl ether in pentane as mobile phase. The solvent was evaporated to yield [<sup>2</sup>H<sub>6</sub>]-6-methyl-5-hepten-2-one (54.5% purity) (1.8 g; 13.6 mmol). MS-EI, *m/z* (%): 43 (100), 44 (28), 59 (25), 113 (23), 75 (21), 45 (18), 72 (14), 58 (14), 114 (13), 41 (12).

(4) Triethyl phosphonoacetate (Sigma-Aldrich) (9.2 g; 41 mmol) and 30 mL of anhydrous diethyl ether were placed into round bottom flask with stir bar and N<sub>2</sub> purge line. The mixture was stirred at -5°C in an ice-salt-water bath. Sodium hydride (60% dispersion in mineral oil, solid-clay; Acros Organics) (1.1 g; 15.8 mmol) was added, and stirred at this temperature until no more bubbles formed. The mixture was warmed to room temperature and stirring continued under N<sub>2</sub> gas for more than 1 h. The final product from previous step in anhydrous diethyl ether was slowly added, and stirred for an additional 30 min under a N<sub>2</sub> gas stream. The reaction flask was capped under N<sub>2</sub> gas, and stirred overnight. Water (20 mL) was added to stop the reaction at 0°C in an ice-water bath. The mixture was stirred at room temperature for 20 min until the organic layer became colorless. The solvent phase was separated, and the aqueous phase was extract with 25 mL of diethyl ether. The combined extract was washed with 10% aqueous NaOH (1 × 20 mL) and saturated aqueous NaCl (2 × 20 mL).

The extract was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and the solvent was removed. The yield of crude [ $^2\text{H}_6$ ]-7-methyl-3-methyl-2,6-octadienoic acid, ethyl ester was 97% (58% purity). The yellow crude oil was purified on silica gel column using 5% diethyl ether in pentane as mobile phase. The final product after solvent removal was 1.71 g (8.5 mmol) with 82% purity. MS-EI (*cis*-isomer),  $m/z$  (%): 75 (100), 129 (51), 44 (40), 45 (29), 101 (26), 43 (23), 128 (20), 39 (15), 157 (15), 82 (14). MS-EI (*trans*-isomer),  $m/z$  (%): 75 (100), 44 (37), 129 (36), 45 (28), 43 (18), 101 (16), 128 (14), 157 (12), 39 (12), 82 (10).

(5) Lithium aluminum hydride (Sigma-Aldrich) (0.6 g; 16 mmol) was prepared in a three necked flask equipped with a  $\text{N}_2$  purge line and stir bar. Anhydrous diethyl ether (20 mL) was added, and the mixture was stirred at room temperature for 15 min. The final product from the previous step in 5 mL of diethyl ether was added drop by drop into the milky grey suspension at  $-50^\circ\text{C}$ . The mixture was further stirred at room temperature for 1 h, and cooled down to  $0^\circ\text{C}$ . Water was added drop by drop to decompose the excess  $\text{LiAlH}_4$ . The reaction solution was acidified to  $\text{pH} < 2$  by using aqueous 4 N  $\text{H}_2\text{SO}_4$  to neutralize lithium hydroxide and break the emulsion. The organic phase was recovered, and the aqueous phase was extracted with diethyl ether. The pooled extract was washed with saturated aqueous  $\text{NaCl}$ , dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated to 10 mL. The extract was purified by vacuum distillation. The yield after solvent removal was 83% (75% purity). The crude oil was further purified by flash column chromatography using 5% diethyl ether in pentane as mobile phase. The final yield was 48% (92.1% total purity) (32.8% purity of **I-36** and 59.3% purity of **I-39**). MS-EI (**I-36**),  $m/z$  (%): 75 (100), 44 (41), 45 (31), 93 (21), 43 (19), 41 (18), 84 (18), 80 (11), 68 (10), 46 (10). MS-EI (**I-39**),  $m/z$  (%): 75 (100), 44 (34), 45 (25), 41 (15), 43 (14), 129 (11), 68 (11), 93 (10), 84 (9), 46 (7).

*[<sup>2</sup>H<sub>6</sub>]-neral (I-30) and [<sup>2</sup>H<sub>6</sub>]-geranial (I-34)*. Labeled citral (mixture of *cis*- and *trans*-isomers) was obtained by oxidation of the corresponding alcohols using the procedure in the literature (Corey and Suggs, 1975). A suspension of pyridinium chlorochromate (Sigma-Aldrich) (1.25 g; 5.8 mmol) in 10 mL CH<sub>2</sub>Cl<sub>2</sub> was prepared in 50-mL vial. The mixture of [<sup>2</sup>H<sub>6</sub>]-nerol (**I-36**) and [<sup>2</sup>H<sub>6</sub>]-geraniol (**I-39**) (0.62g; 3.9 mmol) in 2 mL of CH<sub>2</sub>Cl<sub>2</sub> was added in one portion to the suspension, and stirred for 1.5 h. Diethyl ether (20 mL) was added, and supernatant was decanted. The residue was further extracted with diethyl ether (3 × 10 mL) until the black gum became a granular solid. The pooled ether extract was filtered through a bed of Florisil<sup>®</sup> (Sigma-Aldrich), and then concentrated to about 10 mL. The sample was purified by high vacuum distillation and flash chromatography. The final yield was 73% (74.1% total purity) (21.8% purity of **I-30** and 52.3% purity of **I-34**). MS-EI (**I-30**), *m/z* (%): 75 (100), 44 (63), 45 (48), 94 (37), 43 (26), 85 (25), 65 (23), 95 (21), 41 (17), 84 (16). MS-EI (**I-34**), *m/z* (%): 75 (100), 44 (43), 45 (33), 85 (22), 94 (17), 43 (16), 39 (9), 46 (9), 84 (9), 41 (8).

*[<sup>13</sup>C<sub>2</sub>]-2-Phenethyl acetate (I-37)*. This labeled acetate ester was synthesized followed a published procedure (Furniss et al., 1989). [<sup>13</sup>C<sub>2</sub>]-2-Phenylethanol (**I-43**) (0.43 g; 3.5 mmol), triethylamine (Sigma-Aldrich) (0.43 g; 4.2 mmol), and 20 mL of CH<sub>2</sub>Cl<sub>2</sub> were prepared in a pre-dried 100-mL round bottom flask equipped with stir bar, rubber septum, N<sub>2</sub> purge line, vent needle, and cooled in an ice-water bath at 0°C. Acetyl chloride (Fluka, St. Louis, MO) (0.33 g; 4.2 mmol) in 5 mL of CH<sub>2</sub>Cl<sub>2</sub> was slowly added to the stirring solution, and the mixture was stirred for 10 min under N<sub>2</sub> gas before removing the needle and sealing with a septum. After stirring for 6 h, 20 mL of water was added to stop the reaction. The mixture was stirred until the precipitate was dissolved. The organic phase was separated, and the aqueous phase was

extracted with diethyl ether ( $2 \times 15$  mL). The pooled solvent layer was washed with aqueous 10%  $\text{H}_2\text{SO}_4$  and then with saturated aqueous  $\text{NaHCO}_3$  ( $2 \times 10$  mL). The extract was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and the solvent was removed to obtain the labeled standard (**I-37**). MS-EI,  $m/z$  (%): 106 (100), 43 (61), 92 (19), 107 (10), 105 (8), 79 (5), 51 (5), 66 (5), 52 (4), 78 (4).

*[ $^2\text{H}_3$ ]-Eugenol (I-51)*. The synthesis method was adapted from published procedures (Schneider and Rolando, 1992; Kulkarni et al., 1999) as detailed below.

(1) N,N-Dimethylaniline (Aldrich, St. Louis, MO) (9.6 g; 79 mmol) and aluminum chloride (Sigma-Aldrich, St. Louis, MO) (10.6 g; 80 mmol) were placed in round bottom flask equipped with reflux condenser, stirrer and  $\text{N}_2$  purge line. Anhydrous toluene (5mL) was slowly added. The solution was heated (smoke was generated during addition) and the clear brown solution was stirred for 15 min before adding drop by drop a solution of eugenol (6.56 g; 40 mmol) in 15 mL of toluene. The solution was refluxed at 100-110°C for 2 h. The mixture was allowed to cool, and slowly dropped into 50 mL of ice-cold water with stirring. The brown/gray solution was adjusted to pH 1-2. The toluene layer was extract with diethyl ether ( $3 \times 20$  mL). The combined solvent was washed with aqueous 2 M HCl ( $2 \times 20$  mL), and then extracted with aqueous 2 M NaOH ( $3 \times 15$  mL). The pooled aqueous phase (brown in color) was acidified to pH 1-2 before extraction with diethyl ether ( $3 \times 25$  mL). The extract was dried over anhydrous  $\text{Na}_2\text{SO}_4$  before removal of solvent. The residue was purified on silica gel column using 50% of diethyl ether in pentane as mobile phase. 4-Allycatecol was obtained after the solvent was removed (89% yield). MS-EI,  $m/z$  (%): 137 (100), 166 (20), 122 (14), 39 (13), 94 (12), 77 (10), 51 (10), 138 (9), 66 (7), 65 (7).

(2) A solution of 4-allycatecol (1.5 g; 10 mmol) in ethanolic NaOH (2 M, 10 mL) was prepared in a 50-mL screw cap test tube equipped with stir bar. [ $^2H_3$ ]-Iodomethane (Isotec) (1.45 g; 10 mmol) in 10 mL of ethanol was added drop by drop under a gentle N<sub>2</sub> steam. The tube was sealed with a PTFE-lined cap, and shaken vigorously with periodic venting. The mixture was stirred for 72 h until the reaction was complete. The ethanol was removed using a warm-water bath under a N<sub>2</sub> steam, and then dissolved with 150 mL of water. The mixture was washed with CH<sub>2</sub>Cl<sub>2</sub> (4 × 20 mL) to remove the dimethoxylated compound, and then adjusted to pH < 1. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 20 mL). The pooled solvent phase was extracted with aqueous 1 M NaOH (3 × 15 mL). The aqueous phase was acidified to pH < 1 using aqueous 4 M HCl, and extracted with diethyl ether (3 × 15 mL). The solvent layer was washed with water (2 × 10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated to 10 mL. The mixture was subjected to vacuum distillation to remove any non-volatiles before purification by flash column chromatography (20% CH<sub>2</sub>Cl<sub>2</sub> in pentane). The solvent was evaporated to yield [ $^2H_3$ ]-eugenol (10% yield, 40.2% purity). MS-EI, *m/z* (%): 167 (100), 103 (44), 131 (37), 149 (36), 77 (36), 55 (34), 91 (31), 140 (30), 104 (29), 133 (22).

[ $^2H_6$ ]-Methyleugenol (**I-46**). This labeled standard was a by-product of the aforementioned procedure for the synthesis of [ $^2H_3$ ]-eugenol. To obtain this compound, the mixture solution in pentane (5 mL) was washed with aqueous 1 M NaOH (3 × 5 mL) and then saturated aqueous NaCl (2 × 5 mL) to remove [ $^2H_3$ ]-eugenol. The sample was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the solvent was evaporated (96.7% purity). MS-EI, *m/z* (%): 184 (100), 150 (41), 103 (38), 166 (34), 91 (30), 65 (16), 138 (14), 157 (14), 77 (14), 51 (14).

$[^2\text{H}_3]$ -(*Z*)- and  $[^2\text{H}_3]$ -(*E*)-Isoeugenols (**I-53** and **I-54**). The synthesis method was adapted from published procedures (Ceruti et al., 1994; Scheidig et al., 2007). Ethyltriphenyl phosphonium bromide (Sigma-Aldrich) (1.4 g; 3.6 mmol) was mixed with 2 mL of sodium amide (50 wt. % suspension in toluene; Sigma-Aldrich) (6 mmol), 3 mL of butyllithium (2.0 M solution in cyclohexane; Sigma-Aldrich) (6 mmol), and 10 mL of diethyl ether in a 250-mL round bottom flask equipped with a stir bar. The bright pumpkin orange suspension was stirred for 2.5 h at room temperature.  $[^2\text{H}_3]$ -Vanillin (0.2026 g; 1.31 mmol) dissolved in 5 mL of diethyl ether was added (all at once), and the mixture was stirred for 2 h. The reaction mixture was cooled in an ice-water bath and saturated aqueous  $\text{NH}_4\text{Cl}$  (100 mL) was added and stirred vigorously for 15 min. The organic layer was separated, and the aqueous phase was extract with  $\text{CH}_2\text{Cl}_2$  (3  $\times$  20 mL). The pooled organic phase was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and the solvent was evaporated. The crude sample was purified by flash chromatography using 20%  $\text{CH}_2\text{Cl}_2$  in pentane as mobile phase. The mixture of (*Z*)- and (*E*)-isomers were obtained after removal of solvent (89% yield; 91.1% total purity) (80.0% purity of **I-53** and 11.1% purity of **I-54**). MS-EI (**I-53**),  $m/z$  (%): 167 (100), 77 (35), 149 (33), 103 (33), 91 (30), 55 (30), 131 (26), 104 (19), 133 (17), 121 (16). MS-EI (**I-54**),  $m/z$  (%): 167 (100), 77 (32), 103 (31), 149 (31), 91 (29), 55 (26), 131 (26), 133 (18), 104 (17), 121 (16).

The unlabeled isoeugenols were also synthesized in the same manner to obtain (*Z*)- and (*E*)-isomers (68.1:31.9% purity) for determination of odor detection threshold.

$[^2\text{H}_5]$ -Cinnamaldehyde (**I-48**). The isotope compound was synthesized in four steps using a procedure adapted from the literature (Speed et al., 2004; Meyer and Schreiber, 1994) as detailed below.

(1) [ $^2H_5$ ]-Benzyl alcohol (Isotec, Miamisburg, OH) (0.45 g; 4 mmol) in  $CH_2Cl_2$  (2 mL) was slowly dropped into the solution of pyridinium chlorochromate (Sigma-Aldrich) (1.5 g; 6 mmol) and  $CH_2Cl_2$  (12 mL). The mixture which turned quickly from orange to black was stirred for 90 min. Diethyl ether (20 mL) was added, and the residue was further extracted with diethyl ether ( $3 \times 10$  mL) until the gummy residue became granular solid. The combined extracts were passed through dry Florisil<sup>®</sup> (Sigma-Aldrich) (10 g), and the solvent was removed by  $N_2$  gas to obtain [ $^2H_5$ ]-benzaldehyde (80% yield, 85.1% purity). MS-EI,  $m/z$  (%): 82 (100), 110 (95), 111 (88), 54 (66), 52 (40), 83 (21), 55 (14), 42 (10), 40 (10), 76 (9).

(2) (Carbethoxymethylene)triphenylphosphorane (Sigma-Aldrich) (1.6 g; 4.6 mmol) was dissolved in  $CH_2Cl_2$  (10 mL) under  $N_2$  gas, and the solution was cooled down to  $0^\circ C$  in an ice bath. [ $^2H_5$ ]-Benzaldehyde (0.36 g; 3.2 mmol) in cold  $CH_2Cl_2$  (1 mL) was dropped into the stirred mixture. After stirring 10 min, the mixture was allowed to rise to room temperature, and the solvent was evaporated under  $N_2$  stream. The white residue was extracted with pentane ( $1 \times 10$  mL, then  $2 \times 5$  mL). The combined extracts were filtered through glass wool before removal of solvent. The ethyl-(*E*)-[ $^2H_5$ ]-cinnamate was obtained in a 90% yield and 94.7% purity. MS-EI,  $m/z$  (%): 136 (100), 108 (61), 181 (29), 81 (24), 80 (21), 54 (21), 109 (19), 153 (17), 107 (16), 82 (13).

(3) Ethyl-(*E*)-[ $^2H_5$ ]-cinnamate (0.38 g; 2.2 mmol) in diethyl ether (10 mL) was prepared in three-necked flask equipped with a condenser ( $5^\circ C$ ) and a  $N_2$  purge line. Lithium aluminum hydride (Sigma-Aldrich) (1.5 mL; 1.45 mmol) in diethyl ether (3.5 mL) was added dropwise into the stirred solution. After 2 h, the mixture was cooled down in an ice-water bath, and water (8 mL) was slowly added to decompose the excess  $LiAlH_4$ . The solution was acidified to pH 2 with aqueous 4 M  $H_2SO_4$  to neutralize lithium hydroxide and break the emulsion. The aqueous

phase was further extracted with diethyl ether ( $2 \times 10$  mL). The pooled extracts were washed with saturated aqueous NaCl ( $3 \times 5$  mL), concentrated to 5 mL, and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The purification was performed by vacuum distillation and flash column chromatography on silver nitrate on silica gel using 20% diethyl ether in pentane to remove [<sup>2</sup>H<sub>5</sub>]-benzenepropanol from [<sup>2</sup>H<sub>5</sub>]-cinnamyl alcohol (11% yield, 82.7% purity). MS-EI, *m/z* (%): 97 (100), 96 (97), 139 (83), 110 (59), 83 (55), 82 (38), 95 (34), 119 (33), 81 (30), 55 (28).

(4) [<sup>2</sup>H<sub>5</sub>]-Cinnamyl alcohol (25 mg; 0.18 mmol) and Dess-Martin periodinane (Lancaster, Pelham, NH) (0.11 g; 0.27 mmol) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) in a round bottom flask equipped with stir bar. Wet CH<sub>2</sub>Cl<sub>2</sub> (3.3 μL water in 12.5 mL CH<sub>2</sub>Cl<sub>2</sub>; 0.18 mmol) was added dropwise into the mixture (cloudy white appeared). Almost all the solvent was evaporated using a gentle stream of N<sub>2</sub> gas and a water bath (50°C). Diethyl ether (25 mL) and the mixture of 10% sodium thiosulfate (JT. Baker, Phillipsburg, NJ) (12.5 mL) and saturated aqueous NaHCO<sub>3</sub> (12.5 mL) were added, and stirred vigorously. The aqueous layer was extracted with diethyl ether ( $2 \times 10$  mL). The combined extracts were washed with saturated aqueous NaHCO<sub>3</sub> ( $2 \times 10$  mL), and then saturated aqueous NaCl ( $2 \times 10$  mL). The solution was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and 0.1% equivalent BHT (Sigma-Aldrich) was added to avoid further oxidation before removal of solvent. The yield of [<sup>2</sup>H<sub>5</sub>]-cinnamaldehyde was 87%, and the purity was 80.8%. MS-EI, *m/z* (%): 137 (100), 135 (93), 108 (63), 136 (55), 109 (49), 54 (43), 81 (40), 107 (40), 83 (38), 82 (35).

[<sup>2</sup>H<sub>6</sub>]-Coumarin (**I-56**). Platinum black (Sigma-Aldrich) (5.1 mg) combined with unlabeled coumarin (105.3 mg; 0.72 mmol) were combined with fresh deuterium oxide (99.9 % atom *d*, Sigma-Aldrich) (2 mL; 100 mmol) in pre-dried 10-mL glass ampoule. The mixture was

purged with N<sub>2</sub> for 15 min and the ampoule immediately sealed with a flame torch. After incubation at 120°C for 48 h, the mixture was allowed to cool and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the solvent was evaporated to obtain [<sup>2</sup>H<sub>6</sub>]-coumarin (99% yield, 99.8% purity). MS-EI, *m/z* (%): 124 (100), 152 (76), 96 (38), 94 (37), 66 (25), 123 (17), 151 (13), 54 (13), 62 (10), 52 (10). The chemical structure was also confirmed by <sup>1</sup>H nuclear magnetic resonance (NMR) on a Varian Inova-500 NMR spectrometer (Varian Instruments, Palo Alto, CA) at a proton frequency of 500 MHz. Labeled standard (**I-56**) or unlabeled coumarin (**56**) was prepared ~10<sup>-3</sup> M in CDCl<sub>3</sub> (containing 0.03% v/v TMS; Sigma-Aldrich), and the <sup>1</sup>H NMR spectra of the isotope standard were compared to that of unlabeled coumarin (**Figure 4.1**).

### Stable Isotope Dilution Analysis

The isotopically labeled standards of selected aroma-active compounds were prepared individually in dichloromethane (~1 mg/mL) instead of methanol to avoid decomposition, especially the conversion of aldehydes to acetals. The chemical and isotopic stabilities of all isotopically labeled compounds were confirmed using an aqueous citrate buffer (pH 2.3, 0.12% (w/v) citric acid), which was similar to the cola matrix, for 18 h (**Figures C.1 – C.13**). The appropriate amount of isotope to add in the cola was checked for each standard as well. All isotope standard solutions were added into cola as internal standards before extraction.

***Isolation of limonene and  $\alpha$ -terpineol.*** About five milliliters of cola from the original can were poured into a 20-mL scintillation vial containing 5 mL of diethyl ether to prevent loss of volatiles. The exact weight of cola was recorded as the weight different. The isotope standards were added into the cola layer before extraction. The sample was de-carbonated by

sonication (model 2200; Branson Ultrasonics, Co., Danbury, CT) using an ice-water bath at 10°C for 5 min, and then shaken (DS-500 orbital shaker; VWR Scientific, Batavia, IL) at 200 rpm for 10 min. The separated aroma extract was dried over 1 g of anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated to 0.5 mL before analysis by GC-MS.

***Isolation of other aroma compounds.*** The volatile compounds were extracted from one can of cola in the same manner as previously described in **Chapter 3**. The aroma components were isolated by continuous liquid-liquid extraction (CLLE), and any non-volatiles were removed by solvent assisted flavor evaporation (SAFE) before fractionation into acidic and neutral-basic fractions.

One microliter of the aroma extract was injected into a GC-mass spectrometer (GC-MS) system, consisting of a 6890 GC/5973 mass selective detector (Agilent Technologies, Inc., Santa Clara, CA), in the cold splitless mode (-50°C held for 0.1 min, and then raised at 12°C/s to 250°C) to avoid loss of volatiles in the injection port. Volatile compounds were separated on either Stabilwax<sup>®</sup> (30 m × 0.25 mm i.d. × 0.25 μm df; Restek, Bellefonte, PA) or SAC<sup>TM</sup>-5 (30 m × 0.25 mm i.d. × 0.25 μm df; Supelco, Bellefonte, PA) capillary columns. The initial oven temperature was 35°C. After 5 min, the oven temperature was increased at 4°C/min to the final temperature (225°C for Stabilwax<sup>®</sup> or 240°C for SAC<sup>TM</sup>-5), and held for 20 min. The flow rate of helium carrier gas was 1 mL/min. The mass spectra were performed in full scan mode (35-300 a.m.u., scan rate 5.27 scans/s, interface temperature 280°C, and ionization energy 70 eV).

For quantification, the mass ions for isotopes and unlabeled target compounds were chosen on the basis of uniqueness and relative intensity. The peak areas for selected ions were integrated using MSD ChemStation software (Agilent Technologies, Inc.). The mass of a target

compound was calculated from the mass ratio, area ratio, and GC-MS response factor ( $R_f$ ).  $R_f$  was calculated as the inverse of slope of the calibration plot of area ratio versus mass ratio (equation 4.1), determined by analysis of five levels of standard compound (unlabeled) against the isotope compound (labeled). The  $R^2$  values of linear regressions obtained for calibration plots were greater than 0.9 for all compounds. The concentration of each compound in cola was calculated as equation 4.2. The mean value was determined from four cans of cola from the same code date.

$$R_f = \frac{[\text{area of ion}_t / \text{area of ion}_i]}{[\text{mass}_t / \text{mass}_i]} \quad (4.1)$$

$$\text{concentration}_t = \text{concentration}_i \times R_f \times \left[ \frac{\text{area of ion}_t}{\text{area of ion}_i} \right] \quad (4.2)$$

where:  $i$  = labeled internal standard

$t$  = target compound

### Odor Activity Values

Since water is the major constituent of cola, the odor activity value (OAV) of a compound was calculated as the ratio of its concentration in cola to its odor threshold in water. The odor detection thresholds in water of the aroma-active compounds were obtained from the literature or determined in our lab. The aroma compounds used to determine the threshold were purified by vacuum distillation and/or flash chromatography, and checked for purity by neat injection (hot split 1:50 at 250°C) into a GC equipped with flame ionization detector (FID).

***Determination of odor thresholds in water.*** The odor detection threshold of a compound was determined orthonasally followed published procedures (ASTM Standard, 2004; Watcharananun et al., 2009). The high purity standard of isoborneol (**29**) or (*E*)-cinnamaldehyde

(48) was prepared in methanol, and dissolved in odor-free water. The blank samples were also spiked with methanol in odor-free water. All test samples (50 mL each) were presented in Teflon sniff bottles because this method offers greater sensitivity and less variation (Guadagni et al., 1963). The participants sniffed a set of three samples with one containing the aroma compound. They were asked to select the sample having the strongest odor by the 3-alternative force choice (3-AFC) test. An ascending concentration series consisting of six samples differing by a dilution factor of three per step was tested. The individual best estimate threshold (BET) was calculated as the geometric mean of the highest concentration with an incorrect response and the higher concentration with a correct response. The group BET was calculated as the geometric mean of the individual BET. Number of panelists for evaluation of odor threshold of isoborneol and (*E*)-cinnamaldehyde were 12 (8 females and 4 males; 22-47 years) and 11 (7 females and 4 males; 22-47 years), respectively.

***Determination of odor thresholds in air.*** The relative odor thresholds of (*Z*)- and (*E*)-isoeugenols (53 and 54) in air were evaluated by GCO according to method reported in the literature (Ullrich and Grosch, 1987) using eugenol (51) as an internal standard. The high purity standards of isoeugenols and eugenol were prepared in CH<sub>2</sub>Cl<sub>2</sub>, and diluted stepwise (1:3 v/v). The panelists evaluated each dilution by GCO (described in **Chapter 3**) until they were not able to smell any odor. The highest dilution of each aroma compound was recorded, and the relative odor thresholds of (*Z*)- and (*E*)-isoeugenols were calculated based on that of eugenol (0.2-0.3 ng/L air (ppt); Blank et al., 1989). The group threshold was calculated as the geometric mean of individual thresholds of seven panelists (3 females and 4 males; 21-38 years).

## **Aroma Reconstitution Model**

The cola composition, including pH, soluble solids, and titratable acidity were determined in a de-carbonated (by sonication) cola for three samples with different code dates. The model matrix was prepared on the basis of the information in **Table 4.4** using a sucrose-phosphoric acid solution. The cola base was adjusted to pH 2.31 using aqueous 1 M of NaOH or HCl. The aroma compounds (**Table 4.3**) were purified and their purities determined by GC as described earlier.

The model was constructed based on quantification data from stable isotope dilution analysis (SIDA). The high purity standards were prepared individually in methanol, except octanal, nonanal, and decanal (**13, 16, 21**). High purity standards of these aldehydes were dissolved in pentane to avoid degradation reactions, and diluted in methanol just before preparing the model. The solutions of aroma compounds were spiked into the cola matrix. To determine if the model was similar to the actual product, the model was compared to de-carbonated cola (authentic cola) by sensory descriptive aroma profiling followed the procedure from our previous study (**Chapter 3**). The approval protocol number from the University of Illinois at Urbana-Champaign Institutional Review Board (IRB) for conducting all sensory analysis in this experiment was 11249. Panelists received an additional three hours of refresher training prior to conducting the evaluation. The aroma profile also provided information about the major aroma attributes (terms) of cola and the cola model and how they were similar or how they differed, thus allowing for the possibility of adjusting the model to better match the authentic cola. The model was deemed appropriate for omission studies based on the similarity (or “matching”) of its aroma profile to that of the authentic cola. Ten trained panelists (7 females and 3 males; 23-45 years) participate in this descriptive analysis in individual sensory booths.

***Solid phase microextraction.*** Headspace-solid phase microextraction (HS-SPME) was also used to compare the HS volatiles of authentic cola and those of cola models for the purpose of adjusting the model to better match the authentic cola. Commercial cola or cola models (5 mL) and 1 g of NaCl was prepared in 20-mL screw top headspace vials equipped with Teflon-coated stir bar and a Teflon-lined silicon closure. The sample vial was incubated at 40°C for 10 min at the agitator speed 250 rpm. The SPME fiber (50/30µm DVB/Carboxen<sup>TM</sup>/PDMS StableFlex<sup>TM</sup>; Supelco, Bellefonte, PA) was exposed into HS of the sample vial, and extracted the HS volatiles for 20 min at the same temperature. Then, the aroma volatiles in SPME fiber were desorbed in the hot splitless injection port of GC-MS or GCO at 260°C for 14 min with 4 min GC valve delay. The volatiles were separated on polar capillary column, and the GC-MS parameters were the same as described for quantification by SIDA. The GCO conditions were as previous described in **Chapter 3**.

### **Omission Studies of Aroma Reconstitution Models**

Omission studies were performed by comparing the complete model against the omission models using R-index ranking test following the procedure in the literature (Lorjaroenphon et al., 2008). Potent odorants were grouped based on their similar odor characteristics, properties, or the origins of compounds (**Table 4.5**). The complete model of cola was constructed as described above, and the omission models were prepared by omitting a group of odorants (methanol was spiked instead). All samples (50 mL) were prepared in sniff bottles covered with aluminum foil. The complete model was labeled as control, and the omission models and another set of complete model were labeled with randomly 3-digit codes.

The test samples (omission models and complete model with 3-digit code) were presented to the panelists (n = 28; 19 females and 9 males; 20-48 years) using randomized and balance serving orders. After the panelists evaluated the samples by gently squeezing the bottle and sniffing the expressed air, they were asked to rank the test samples on how similar they were to the control (complete model). The R-index values were calculated as the percentage of times the sample was ranked less similar to the complete model using the John Brown computation (O'Mahony, 1992). These values were compared to the critical value (n = 28) for two-tailed test at  $\alpha = 0.05$  to determine if differences were significant.

Additional omission studies were conducted for individual components of a group whenever a group omission model was determined to be significantly different from the complete model. Twenty six panelists (18 females and 8 males; 20-48 years) evaluated the test samples against the control sample (complete model) in the same manner as previously described. In addition, the significantly different group omission model was subjected to sensory descriptive (aroma profile) analysis by an 8-member trained panel (5 females and 3 males; 26-45 years). A paired t-test performed by SAS<sup>®</sup> program (SAS institute Inc., Cary, NC) was used to compare between the attribute means of the complete model and those of the omission model.

## **V. RESULTS AND DISCUSSION**

Based on results from the previous study described in **Chapter 3** cola A was chosen as the representative cola-flavored carbonated beverage for the present study because it contained more moderately and highly potent odorants than in colas B and C. The odor-active compounds from aroma extract dilution analysis (AEDA) were selected for calculation of odor activity values (OAVs) based on high potency (high flavor dilution (FD) factor). Some less potent

odorants were also quantified. In particular, limonene (**8**) and  $\alpha$ -terpineol (**31**) with low FD factors were included in this experiment since these two odorants were present at highest abundance in all colas, so they might exert some influence on the perception of cola aroma.

### Quantification by Stable Isotope Dilution Analysis

In the present study, stable isotope dilution analysis (SIDA) was used for accurate quantification. The SIDA method is highly accurate since isotopically labeled compounds are used as internal standards. The chemical and physical properties of labeled isotopes are the same as those of the target compounds. This means that losses of labeled (isotope) and unlabeled (analyte) compounds during extraction were the same and their ratio to one another stay constant. All isotopes used in this study (**Figure 4.2**) were labeled with deuterium, except for phenylacetic acid (**I-57**) and its derivatives (**I-37** and **I-43**) which were labeled with  $^{13}\text{C}$ . Among synthesized isotopes, [ $^2\text{H}_6$ ]-coumarin (**I-56**) was prepared by exchanging the hydrogen atoms with deuterium atoms. The electron-impact (EI) mass spectrum of **I-56** indicated that six atoms of hydrogen were substituted, but it was important to confirm the chemical structure of this isotope by nuclear magnetic resonance (NMR). The  $^1\text{H}$  NMR spectra of **I-56** showed no signal (**Figure 4.1**), except for internal standard (TMS) and solvent ( $\text{CDCl}_3$ ) peaks. This confirmed that all hydrogen atoms were nearly completely replaced by deuterium atoms.

The concentrations of selected odorants identified in the cola are shown in **Table 4.1**. Among thirty aroma compounds, limonene (**8**) was in highest abundance, followed by  $\alpha$ -terpineol (**31**), and acetic acid (**19**). Neral (**30**), geranial (**34**), and  $\beta$ -damascenone (**40**) were present at levels below their limits of detection by either full scan or SIM modes of EI-GC-MS.

## Odor Detection Thresholds

Since an aroma compound can interact with other components in various ways, its odor threshold determined in different mediums will not be the same (Mistry et al., 1997). It is important to use odor thresholds in the same matrix as the food sample for accurate OAV calculation. Odor thresholds in water were used for calculate OAVs of potent odorants in cola since the matrix components of cola should not greatly affect the odor detection thresholds for most odorants. A previous study of flavor release from sucrose solution indicated that flavor release of the thirteen aroma compounds studied, including compounds such as linalool and  $\beta$ -damascenone in water and those in aqueous 20% (w/v) sucrose solution were not significantly different (Rabe et al., 2003). On the other hand, odor thresholds of some odorants are affected by pH of the matrix, for example Furaneol<sup>TM</sup> (Buttery et al., 1995). Some weak acid compounds, such as butanoic acid (**26**) and 3-methylbutanoic acid (**28**), are most volatile at pH values below their pKa values.

Most odor thresholds in water of potent odorants were obtained from the literature (**Table 4.1**). Two of them were evaluated in our lab using high purity standard compounds (98.7% of isoborneol (**29**) and 99.9+% of (*E*)-cinnamaldehyde (**48**)). It is important to note that the isoborneol standard consisted of a racemic mixture (49.1%:50.9%) of the two enantiomers. As we know that enantiomers can have different odor thresholds, the configuration of chiral compound should be considered. Among chiral aroma compounds in cola, linalool (**22**) has the greatest difference (more than nine fold) between the odor detection thresholds of the two enantiomers.

The aroma compound used to determine the odor threshold must be highly pure before testing. Sometimes this is difficult or nearly impossible, such as in the case of the two isomers of

isoeugenol, which could not be separated by using flash chromatography or high pressure flash chromatography, so determination of their odor thresholds in water could not be determined. Their odor thresholds in air should, however, give some indication of how much they influence cola aroma. Unfortunately, these values are not available in the literature. For this reason, the relative odor thresholds in air of (*Z*)- and (*E*)-isoeugenols (**53** and **54**) were determined by GC-olfactometry (GCO) in the present study.

Eugenol (**51**), which has an odor threshold in air of 0.2-0.3 ng/L air (ppt) (Blank et al., 1989), was used as an internal standard. The relative odor threshold in air of (*Z*)-isoeugenol (0.4-0.6 ng/L air; ppt) was about two fold higher than that of eugenol, while the value of (*E*)-isoeugenol (0.1-0.2 ng/L air; ppt) was lower (**Table 4.2**).

### Odor Activity Values

OAVs specific aroma-active compounds in cola were calculated as the ratio of the concentration the aroma compounds in cola to their odor detection thresholds in water (**Table 4.1**). In cola-flavored carbonated beverage, limonene (**8**) had the highest OAV followed by 1,8-cineol (**9**), octanal (**13**), and linalool (**22**). OAVs for some potent odorants from **Chapter 3** could not be calculated due to lack of concentration data (e.g., neral (**30**), geranial (**34**), and  $\beta$ -damascenone (**40**)) or their odor detection thresholds in water were unavailable (e.g., (*Z*)-isoeugenol (**53**) and (*E*)-isoeugenol (**54**)). Additionally, the OAVs of borneol (**32**) were reported as the values of (+)- and (-)-isomers because the enantiomeric excess of borneol in cola could not be determined (**Chapter 3**).

When the enantiomeric composition was taken into account, the OAVs of (*S*)-(-)-limonene (**8a**; *l*-isomer) and (*R*)-(+)-limonene (**8b**; *d*-isomer) decreased to 0.3 and 8.1,

respectively, while OAV of (*R*)-(-)-linalool (**22a**) increased up to 60.1. The other enantiomer pairs ((*S*)-(+)- and (*R*)-(-)-4-terpineols (**25a** and **25b**); (*S*)-(-)- and (*R*)-(+)- $\alpha$ -terpineols (**31a** and **31b**)) showed lower OAVs than the racemic mixtures (**25** and **31**). Furthermore, their values decreased to below one.

The compounds 1,8-cineol (**9**), (*R*)-(-)-linalool (**22a**), and octanal (**13**) were the most potent odorants which contribute to the overall aroma of cola. Compounds of moderate potency included nonanal (**16**), (*S*)-(+)-linalool (**22b**), decanal (**21**), and (*R*)-(+)-limonene (**8b**).

1,8-Cineol (**9**) is one of the most odor-active compounds in orange peel oil (Fischer et al., 2008), and it was reported as a secondary degradation product of limonene and  $\alpha$ -terpineol by acid-catalyzed reactions (Clark and Chamblee, 1992) and was the second most abundant volatile compound found in cinnamon oil distilled from root bark (Senanayake et al., 1978).

The higher potency of octanal (**13**) by means of OAV calculation compared to AEDA was due to its low odor detection threshold. This aldehyde was reported as the highest intensity aroma compound in orange oil (Högnadóttir and Rouseff, 2003). Additionally, octanal was reported as a moderately potent odorant in orange oil (Fischer et al., 2008). Octanal, nonanal, and decanal (**13**, **16**, **21**) were also of medium potency in fresh lemon oil (Schieberle and Grosch, 1988).

As mentioned in **Chapter 3**, linalool (**22**) is an important potent odorant of lemon oil (Schieberle and Grosch, 1988) and orange peel oil (Fischer et al., 2008). This compound is the most abundant volatile constituent in coriander oil (Kerrola and Kallio, 1993; Gil et al., 2002; Ravi et al., 2007) and neroli oil (Toyoda et al., 1993). Furthermore, linalool is a dominant alcohol in orange oil (Sawamur et al., 2005). (*S*)-(+)-Linalool (**22b**) comprises 60-70% of coriander oil, while the (*R*)-(-)-enantiomer (**22a**) is predominant in neroli oil (Opdyke, 1975).

The higher potency on the basis of OAV of (*R*)-(-)-linalool over (*S*)-(+)-linalool in cola flavor was due to the lower odor detection threshold of the (*R*)-(-)-enantiomer.

Limonene (**8**) is the major volatile component of lemon oil (Coppella and Barton, 1987; Njoroge et al., 1994), lime peel oil (Minh et al., 2002) and orange oil (Högnadóttir and Rouseff, 2003). It was also present at highest concentration in cola. The majority of enantiomer of limonene in cola was the (*R*)-(+)-isomer (**8b**) accounting for 97% (**Chapter 3**). Its odor threshold is very high compared to the other aroma-active compounds found in cola, so it is interesting to note that the high potency of this enantiomer was due to its high concentration, not its low odor detection threshold.

The other important potent odorants in cola A were vanillin (**58**), borneol (**32**), coumarin (**56**), and eugenol (**51**). The lower potencies, by mean of OAV calculation, of coumarin and eugenol compared to the results from AEDA are due to the low volatilities of these semi-volatile compounds. For example, the odor threshold in air of eugenol is 0.2-0.3 ng/L air (ppt) (Blank et al., 1989), while its threshold in water is 6 ppb (Buttery et al., 1987). Since the volatility of (*Z*)- and (*E*)-isoeugenols (**53** and **54**) are as low as eugenol, the potency of these two isomers in cola may be low even if their odor thresholds in air were particularly low (**Table 4.2**). Besides, the OAV of coumarin was less than that of vanillin, but the intensity of coumarin is about three times higher than vanillin (Abernethy, 1969).

These OAV results were also supported by sensory descriptive analysis from **Chapter 3**. The results from the present study indicate the potent odorants in cola. There are, however, limitations of the GCO technique (elution of single peak of aroma extract with no interaction information), OAV calculation (accurate odor threshold in actual food matrix) and mixture suppression or synergy, which have to be considered for aroma analysis. Sensory studies of

aroma compounds in a model cola system can be used to verify the analytical data and correct for those limitations.

### **Aroma Reconstitution Models**

All aroma compounds which had OAVs higher than one in **Table 4.1** (nos. **8, 9, 13, 16, 21, 22, 25, 31, 32, 51, 56, and 58**) were used to construct the cola aroma reconstitution model. Some odorants which had OAVs lower than one (are not considered as aroma-active compounds), were included in the model reconstitution since they could impact cola flavor or flavor release/partitioning. In this case, isoborneol (**29**), nerol (**36**), geraniol (**39**), guaiacol (**41**), methyleugenol (**46**), (*E*)-cinnamaldehyde (**48**), (*Z*)-isoeugenol (**53**), and (*E*)-isoeugenol (**54**) which had medium or high FD factors in colas (**Chapter 3**) were used to prepare the cola aroma model.

The high purity aroma compounds listed in **Table 4.3** were used to prepare the model. The purities of the aroma compounds, purified by distillation and/or flash column chromatography, were more than 99%, except for isoborneol (**29**; 98.7%; plus 1.3% of borneol) and (+)-borneol (**32b**, 96.5%; plus 3.5% of isoborneol). Isoeugenol was present as a mixture of (*Z*)- and (*E*)-isomers (**53** and **54**). (*R*)-(+)-Limonene (**8b**) was used to prepare the cola model because it is present at 97% enantiomer excess in cola (**Chapter 3**), while isoborneol (**29**) and borneol (**32**) were added as racemic mixtures since their enantiomeric distributions could not be evaluated in commercial cola (**Chapter 3**). It is, however, important to note that linalool (**22**), 4-terpineol (**25**), and  $\alpha$ -terpineol (**31**) used in this experiment had different enantiomeric ratios reported for cola A in **Chapter 3**. These standard compounds were used to construct the cola model based on quantification data determined by SIDA.

The chemical composition and pH of commercial cola were examined after de-carbonated by sonication. The mean values were pH = 2.31, soluble solids = 10.73°Brix, and titratable acid = 0.1226 g of citric acid/100 mL or 0.0625 g of phosphoric acid/100 mL (**Table 4.4**). The variation was slight among three different code dates of cola, and the above values are close to the values published by Coca-Cola's scientists for acidity of 0.1246 % (w/v) and pH value of 2.4 (Clos et al., 2008). Soluble solids contents were 11.1°Brix for original Coca-Cola and 11.5°Brix for Pepsi-Cola (Liu and He, 2007). The composition in this table was used to make the matrix of cola model (sucrose-phosphoric acid solution). This cola base was odorless, and it was not different from odor-free water.

The actual concentration of each aroma compound used to construct the cola aroma reconstitution model is shown in **Table 4.3**. The “original” model prepared from cola matrix and high purity standards based on analytical data had a characteristic cola aroma, but could be distinguished from the authentic sample in terms of the intensity of some aroma descriptors. In particular, the herbal and pine attributes of “original” cola model were significantly higher than those of commercial cola (**Figure 4.3, Table D.2**). The differences between the two aroma profiles may cause by: 1) matrix or 2) aroma volatiles and/or balance of odorants above the headspace. To confirm that the synthetic cola base was comparable to the authentic cola base, an odorless matrix was prepared from commercial cola. The aroma volatiles of de-carbonated authentic cola were removed by diethyl ether extraction (3 times; 1 h each) followed by solvent assisted flavor evaporation (SAFE) for 8 h. The nonvolatile residue was then reconstituted with odor-free water to its original composition. The procedure was repeated to obtain the odorless authentic matrix. By comparison of the overall aroma, the odor characteristic of cola model

obtained from this matrix was similar to that of cola model prepared from synthetic base. It was thus concluded that the synthetic matrix was identical to commercial cola base.

Another possible reason was verified by using solid phase microextraction (SPME). The headspace (HS) aromas of authentic cola and model determined by GCO had similar profiles, except for  $\beta$ -damascenone (**40**) which was not detected in the model. The results from GC-MS also indicated that the HS concentrations of some aroma-active compounds in model were different from those in commercial cola. For example, the HS concentration of linalool (**22**) in the model was higher than authentic cola. Moreover, the high purity standard of linalool contained a higher percentage of the (*R*)-(-)-enantiomer (**22a**), which has a lower odor threshold than the (*S*)-(+)- enantiomer (**22b**) (Padrayuttawat et al., 1997), than the original cola reported in **Chapter 3**. Thus, the concentration of nonanal (**16**), linalool (**22**), and nerol (**36**) above HS were adjusted one by one to better match (within 0.5 fold of the HS concentration of commercial cola), and this model was designated as “re-balanced”. Based on HS data, (*R*)-(+)-limonene (**8b**) and (*E*)-cinnamaldehyde (**48**) had to be increased by about 3 and 5 folds, respectively. Unfortunately, the adjusted models seemed obviously different from the authentic cola. They were overwhelming high in piney or cinnamon odors. The appropriate amount of these two aroma components were further adjusted by sensory analysis. Furthermore, concentration of vanillin, which could not be detected by SPME on GC-MS, was increased. The unquantifiable aroma compounds which had high FD factors, including  $\beta$ -damascenone (**40**), Furaneol<sup>TM</sup> (**47**), and sotolon (**52**), were also evaluated by addition to model in an attempt to improve the overall aroma. Each of them was added at odor threshold level or increased up to 10 folds their threshold, but they were not included in the re-balanced model since they caused no improvement or had a negative effect. The actual concentration of each aroma compound in the

re-balanced model is reported in **Table 4.3**. Based on sensory descriptive analysis, the aroma profile of the model did not differ from that of the authentic cola (**Figure 4.3, Table D.2**). Therefore, this model was used as the complete model for the omission study.

### **Omission Studies**

Omission studies of the cola aroma reconstitution models involved the omission of various groups of compounds instead of single odorants. The 20 high purity standard compounds used to construct the cola aroma reconstitution model were categorized into 5 groups (**Table 4.5**). (*R*)-(+)-Limonene (**8b**), linalool (**22**), nerol (**36**), and geraniol (**39**) which had odor characteristic of citrus/floral perceived by GCO (**Chapter 3**) comprised group 1. The aroma compounds in group 2, including 1,8-cineol (**9**), 4-terpineol (**25**), isoborneol (**29**),  $\alpha$ -terpineol (**31**), and borneol (**32a** and **32b**) had similar herbal/cooling notes as determined by GCO (**Chapter 3**). Meanwhile, group 5 consisted of methyleugenol (**46**), (*E*)-cinnamaldehyde (**48**), eugenol (**51**), (*Z*)- and (*E*)-isoeugenols (**53** and **54**) were designated as the brown spice group according to the aroma characters identified during descriptive analysis in **Chapter 3**. Furthermore, eugenol is not only present in nutmeg oil, but it is also a major volatile in cinnamon oil distilled from leaf (Senanayake et al., 1978; Huang et al., 2006). Guaiacol (**41**) was classified in group 4 along with coumarin (**56**) and vanillin (**58**) because of the origin of these compounds in vanilla bean (Pérez-Silva et al., 2006). Octanal (**13**), nonanal (**16**), and decanal (**21**) were categorized in group 3 as the aldehyde group due to their similar properties. These three aliphatic straight-chain aldehydes are known to cross-adapt because of their similar odor qualities (Kurtz et al., 2010), and they also share the same I7 mammalian odorant receptors (Zhao et al., 1998).

Omission models were compared against the complete model by sensory evaluation. There are several sensory techniques used to discriminate the omission models from the complete model, for example duo-trio test (Czerny et al., 1999), triangle test (Christlbauer and Schieberle, 2011), rating scale from extremely different to extremely similar (Dharmawan et al., 2009), descriptive analysis (House and Acree, 2002), and R-index ranking test (Lorjaroenphon et al., 2008). The latter method is superior to general different tests because the R-index ranking test can provide the degree of difference among samples in a single experiment (O'Mahony, 1992). In addition, R-index ranking test can reduce the error of numerical estimate of rating scale testing, and John Brown's R-index computation is not tough to perform (O'Mahony, 1992). Ranking test is also appropriate for untrained panelists (O'Mahony, 1986).

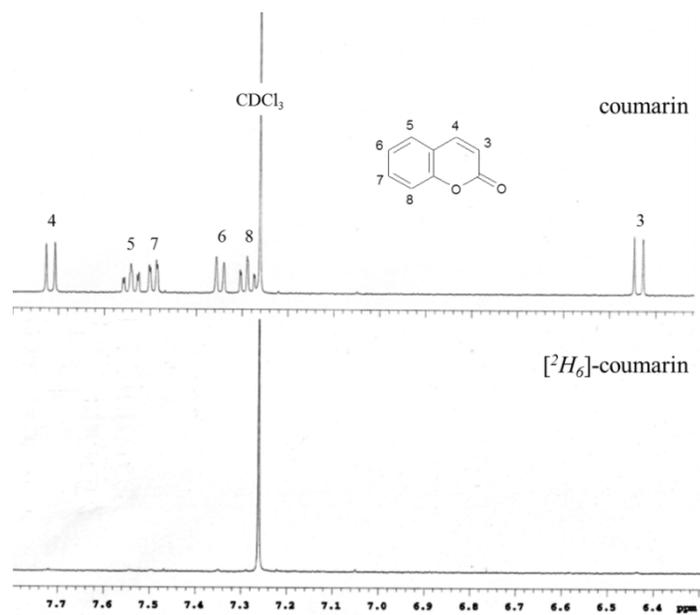
The R-index results revealed that the group 5 omission model was statistically different from the complete model (**Table 4.5**). This indicated that methyleugenol (**46**), (*E*)-cinnamaldehyde (**48**), eugenol (**51**), (*Z*)- and (*E*)-isoeugenols (**53** and **54**) were the most important aroma compounds which contribute to cola flavor. However, omission models based on omitting each of these compounds singularly did not differ from the complete model (**Table 4.6**). This means that the omission of the individual odorants could not be recognized. Food flavor is a result of several odorants, and a changed in odor quality of intensity caused by omission of a single or sometimes even several odorants is usually hard to detect (Grosch, 2001). Additionally, the above results support previous studies where it was shown that sensory panels could not distinguish a single compound in mixtures which contained more than 4 aroma components (Laing, 1994; Laing et al., 1994).

The additional descriptive analysis of aroma (by nose) was also performed to compare between the group 5 omission model (brown spice) and the complete model. The two models

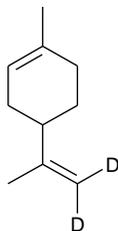
could not be differentiated by the sensory descriptive analysis results (**Figure 4.4, Table D.3**). The complexity of cola flavor and cross-adaptation might be the reasons to explain this result. The omission model contained guaiacol (**41**) and coumarin (**56**) which were described as having a spicy note. Not only similar odor quality (Gottfried et al., 2006), but also similar chemical structure (Pierce et al., 1996) can result in cross-adaptation. Chemical structure of guaiacol consists of -OH and -OCH<sub>3</sub> functional groups on the six-member ring which similar to the structure of eugenol (**51**). Additionally, it could be concluded that R-index ranking test is more powerful than descriptive analysis to discriminate the omission models from the complete model.

In summary, the cola aroma reconstitution model was successfully mimicked based on the analytical data and sensory analysis results. The omission studies also indicated the impact of a “brown spice” group of aroma compounds on the overall aroma of cola. Characterization of key aroma-active compounds by application of OAVs and omission studies is useful for the investigation of aroma changes in colas during storage at various temperatures as described in **Chapter 5**.

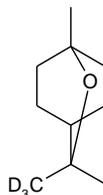
## VI. FIGURES AND TABLES



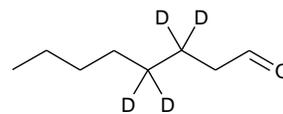
**Figure 4.1**  $^1\text{H}$  NMR spectra of coumarin and  $[\text{}^2\text{H}_6]\text{-coumarin}$  in  $\text{CDCl}_3$  (contains TMS as internal standard).



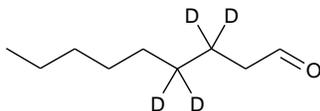
**I-8<sup>a</sup>** [<sup>2</sup>H<sub>2</sub>]-limonene



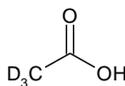
**I-9** [<sup>2</sup>H<sub>3</sub>]-1,8-cineol



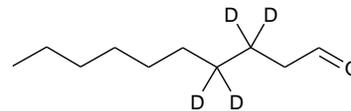
**I-13** [<sup>2</sup>H<sub>4</sub>]-octanal



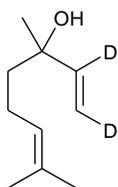
**I-16** [<sup>2</sup>H<sub>4</sub>]-nonanal



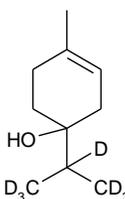
**I-19** [<sup>2</sup>H<sub>3</sub>]-acetic acid



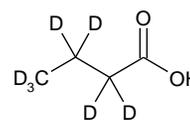
**I-21** [<sup>2</sup>H<sub>4</sub>]-decanal



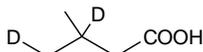
**I-22** [<sup>2</sup>H<sub>2</sub>]-linalool



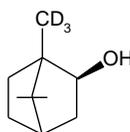
**I-25** [<sup>2</sup>H<sub>7</sub>]-4-terpineol



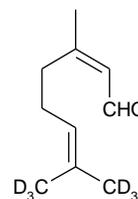
**I-26** [<sup>2</sup>H<sub>7</sub>]-butanoic acid



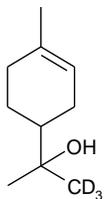
**I-28** [<sup>2</sup>H<sub>2</sub>]-3-methylbutanoic acid



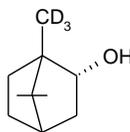
**I-29** [<sup>2</sup>H<sub>3</sub>]-isoborneol



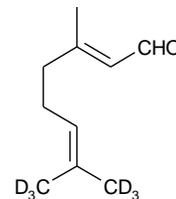
**I-30** [<sup>2</sup>H<sub>6</sub>]-neral



**I-31** [<sup>2</sup>H<sub>3</sub>]- $\alpha$ -terpineol



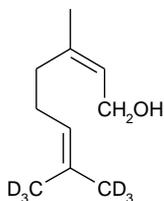
**I-32** [<sup>2</sup>H<sub>3</sub>]-borneol



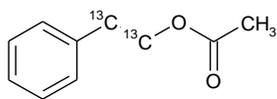
**I-34** [<sup>2</sup>H<sub>6</sub>]-geranial

**Figure 4.2** Chemical structures of isotope standards.

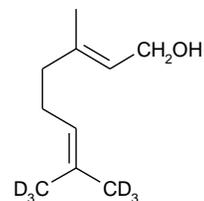
<sup>a</sup>The letter “I” means isotopically labeled compound followed by the numbers which correspond to those in **Tables 4.1 – 4.2** and **Chapter 3**.



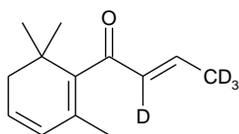
**I-36** [ $^2\text{H}_6$ ]-nerol



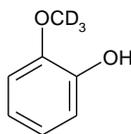
**I-37** [ $^{13}\text{C}_2$ ]-2-phenethyl acetate



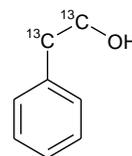
**I-39** [ $^2\text{H}_6$ ]-geraniol



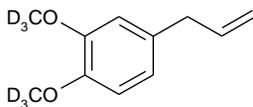
**I-40** [ $^2\text{H}_4$ ]- $\beta$ -damascenone



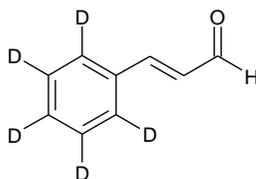
**I-41** [ $^2\text{H}_3$ ]-guaiacol



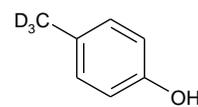
**I-43** [ $^{13}\text{C}_2$ ]-2-phenylethanol



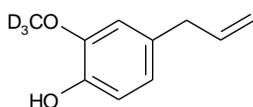
**I-46** [ $^2\text{H}_6$ ]-methyl Eugenol



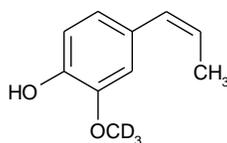
**I-48** [ $^2\text{H}_5$ ]-cinnamaldehyde



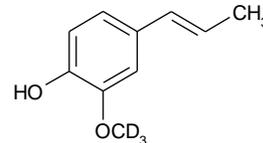
**I-49** [ $^2\text{H}_3$ ]-*p*-cresol



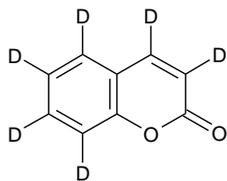
**I-51** [ $^2\text{H}_3$ ]-eugenol



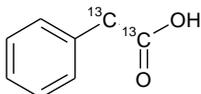
**I-53** [ $^2\text{H}_3$ ]-(*Z*)-isoeugenol



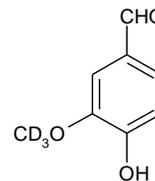
**I-54** [ $^2\text{H}_3$ ]-(*E*)-isoeugenol



**I-56** [ $^2\text{H}_6$ ]-coumarin

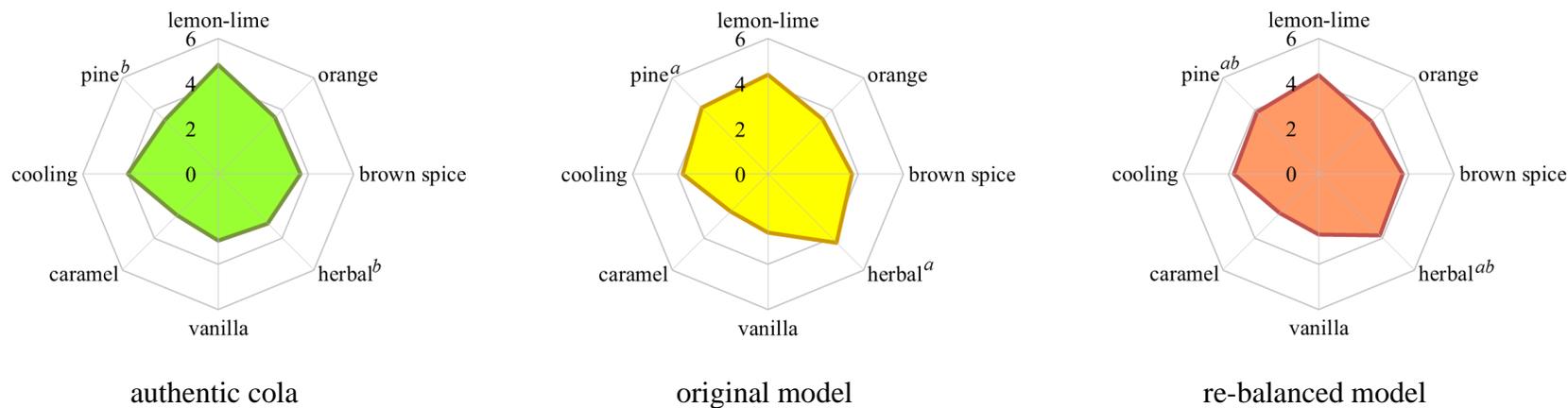


**I-57** [ $^{13}\text{C}_2$ ]-phenylacetic acid

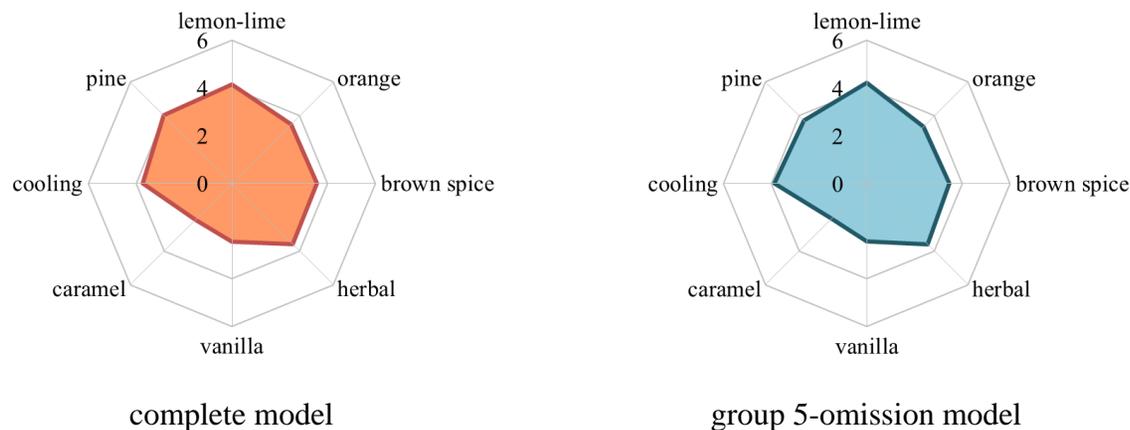


**I-58** [ $^2\text{H}_3$ ]-vanillin

**Figure 4.2** (continued)



**Figure 4.3** Aroma (by nose) profiles by mean of sensory descriptive analysis of cola models compared to authentic cola. Attributes followed by different letters are significantly different ( $p$ -value < 0.05). Averages of intensities correspond to those in **Table D.2** ( $n = 10$ ; 7 females and 3 males; 23-45 years).



**Figure 4.4** Aroma (by nose) profiles of group 5-omission model compared to complete model. Averages of intensities correspond to those in **Table D.3** ( $n = 8$ ; 5 females and 3 males; 26-45 years).

**Table 4.1** Concentrations, odor detection thresholds, and odor activity values of selected odorants identified in cola A.

no. <sup>a</sup>	compound	enantiomeric excess <sup>b</sup>	selected ion (m/z)		R <sub>f</sub> <sup>c</sup>	concentration (ng/g; ppb) <sup>d</sup>	threshold <sup>f</sup>		OAV <sup>x</sup>
			labeled	unlabeled			(ppb)		
<b>8</b>	limonene		138	136	0.98 (0.99+)	10,000	10	<i>i</i>	1,000
<b>8a</b>	<i>(S)</i> -(-)-limonene / <i>l</i> -	2.8				279	1,040	<i>j</i>	0.3
<b>8b</b>	<i>(R)</i> -(+)-limonene / <i>d</i> -	97.2				9,740	1,200	<i>j</i>	8.1
<b>9</b>	1,8-cineole		157	154	0.97 (0.99+)	104	1.3	<i>i</i>	79.8
<b>13</b>	octanal		114	110	0.49 (0.99+)	34.5	0.7	<i>k</i>	49.3
<b>16</b>	nonanal		116	114	0.44 (0.99)	12.8	1	<i>k</i>	12.8
<b>19</b>	acetic acid		63	60	0.95 (0.94)	3,390	22,000	<i>l</i>	0.2
<b>21</b>	decanal		130	128	1.92 (0.99+)	17.5	2	<i>k</i>	8.8
<b>22</b>	linalool		123	121	1.01 (0.99+)	140	6	<i>m</i>	23.2
<b>22a</b>	<i>(R)</i> -(-)-linalool / <i>l</i> -	34.5				48.1	0.8	<i>j</i>	60.1
<b>22b</b>	<i>(S)</i> -(+)-linalool / <i>d</i> -	65.5				91.4	7.4	<i>j</i>	12.4
<b>25</b>	4-terpineol		75	71	0.68 (0.99+)	708	340	<i>i</i>	2.1
<b>25a</b>	<i>(S)</i> -(+)-4-terpinenol	51.0				361	1,290	<i>j</i>	0.3
<b>25b</b>	<i>(R)</i> -(-)-4-terpinenol	49.0				347	1,190	<i>j</i>	0.3
<b>26</b>	butanoic acid		63	60	1.65 (0.91)	11.5	240	<i>l</i>	0.0
<b>28</b>	3-methylbutanoic acid		89	87	0.86 (0.99+)	2.8	250	<i>n</i>	0.0

**Table 4.1** (continued)

no. <sup>a</sup>	compound	enantiomeric excess <sup>b</sup>	selected ion (m/z)		R <sub>f</sub> <sup>c</sup>	concentration (ng/g; ppb) <sup>d</sup>	threshold <sup>f</sup>		OAV <sup>x</sup>
			labeled	unlabeled			(ppb)		
<b>29</b>	isoborneol		113	110	1.07 (0.99+)	15.1	16.4	<i>v, w</i>	0.9
<b>30</b>	neral		75	69	0.68 (0.99+)	< 10 <sup>e</sup>	30	<i>o</i>	-
<b>31</b>	α-terpineol		62	59	0.91 (0.99+)	4,200	350	<i>i</i>	12.0
<b>31a</b>	<i>(S)</i> -(-)-α-terpineol	55.1				2,320	9,180	<i>j</i>	0.3
<b>31b</b>	<i>(R)</i> -(+)-α-terpineol	44.9				1,890	6,800	<i>j</i>	0.3
<b>32</b>	borneol		113	110	0.83 (0.99+)	191	180 <sup>g</sup> / 80 <sup>h</sup>	<i>j</i>	1.1 <sup>g</sup> / 2.4 <sup>h</sup>
<b>34</b>	geranial		75	69	0.69 (0.99+)	< 20 <sup>e</sup>	32	<i>n</i>	-
<b>36</b>	nerol		75	69	0.68 (0.99+)	16.9	300	<i>p</i>	0.1
<b>37</b>	2-phenethyl acetate		106	104	1.00 (0.90)	4.8	20	<i>q</i>	0.2
<b>39</b>	geraniol		75	69	0.96 (0.99+)	23.9	40	<i>r</i>	0.6
<b>40</b>	β-damascenone		73	69	1.04 (0.99)	< 10 <sup>e</sup>	0.002	<i>o</i>	-
<b>41</b>	guaiacol		127	124	1.49 (0.99+)	1.5	3	<i>r</i>	0.5
<b>43</b>	2-phenylethanol		124	122	0.27 (0.98)	3.7	1,100	<i>r</i>	0.0
<b>46</b>	methyleugenol		184	178	1.04 (0.99+)	8.6	68	<i>i</i>	0.1
<b>48</b>	<i>(E)</i> -cinnamaldehyde		137	131	0.57 (0.99+)	808	1,180	<i>v</i>	0.7
<b>49</b>	<i>p</i> -cresol		111	108	1.27 (0.99+)	0.9	55	<i>k</i>	0.0

**Table 4.1** (continued)

no. <sup>a</sup>	compound	enantiomeric excess <sup>b</sup>	selected ion (m/z)		R <sub>f</sub> <sup>c</sup>	concentration (ng/g; ppb) <sup>d</sup>	threshold <sup>f</sup>		OAV <sup>x</sup>
			labeled	unlabeled			(ppb)		
<b>51</b>	eugenol		167	164	1.06 (0.99)	6.6	6	<sup>r</sup>	1.1
<b>53</b>	( <i>Z</i> )-isoeugenol		167	164	0.67 (0.99+)	0.3	-		-
<b>54</b>	( <i>E</i> )-isoeugenol		167	164	2.22 (0.99+)	2.6	-		-
<b>56</b>	coumarin		124	118	0.85 (0.99+)	46.5	25	<sup>s</sup>	1.9
<b>57</b>	phenylacetic acid		138	136	1.45 (0.99+)	0.8	1,000	<sup>t</sup>	0.0
<b>58</b>	vanillin		155	152	1.25 (0.99+)	95.1	25	<sup>u</sup>	3.8

<sup>a</sup>Numbers correspond to those in **Tables 4.2, 4.3, 4.5, 4.6, Figure 4.2** and **Chapter 3**, and the letters after numbers indicated the elution order of enantiomers from the InertCap™ CHIRAMIX column. <sup>b</sup>Expressed as percentage from **Chapter 3**. <sup>c</sup>Response factor and the R-square of the calibration plot was given in parenthesis. <sup>d</sup>Calculated from peak area on Stabilwax® column, except borneol (quantified on SAC™-5 column) (n = 4). <sup>e</sup>Not detected (reported as the concentrations of spiked isotope standards). <sup>f</sup>Odor detection thresholds in water. <sup>g, h</sup>Enantiomers of (+)- and (-)-borneols, respectively. <sup>i</sup>Buttery et al., 1974. <sup>j</sup>Padrayuttawat et al., 1997. <sup>k</sup>Buttery et al., 1988. <sup>l</sup>Buttery and Ling, 1998. <sup>m</sup>Guadagni et al., 1966. <sup>n</sup>Buttery et al., 1990. <sup>o</sup>Buttery et al., 1989. <sup>p</sup>Ohloff, 1978. <sup>q</sup>Liu et al., 2004. <sup>r</sup>Buttery et al., 1987. <sup>s</sup>Masanetz and Grosch, 1998a. <sup>t</sup>Rychlik et al., 1998. <sup>u</sup>Semmelroch et al., 1995. <sup>v</sup>Determined in our lab. <sup>w</sup>Racemic mixture of *d*- and *l*-isomers. <sup>x</sup>Odor activity values.

**Table 4.2** Relative odor detection thresholds in air of isoeugenols determined by GCO.

no. <sup>a</sup>	compound	relative odor detection threshold in air (ng/L air; ppt) <sup>b</sup>
<b>53</b>	( <i>Z</i> )-isoeugenol	0.4 – 0.6
<b>54</b>	( <i>E</i> )-isoeugenol	0.1 – 0.2

<sup>a</sup>Numbers correspond to those in **Tables 4.1, 4.3, 4.5, 4.6, Figure 4.2**, and **Chapter 3**. <sup>b</sup>Using eugenol (**51**) as internal standard.

**Table 4.3** Concentration of high purity standard compounds used in cola models.

no. <sup>a</sup>	compound	purity (%)	enantiomeric excess <sup>e</sup>	concentration (ng/g; ppb)	
				original	re-balanced
<b>8b</b>	<i>(R)</i> -(+)-limonene	99.5		10,100	5,050 <sup>g</sup>
<b>9</b>	1,8-cineole	99.3		101	101
<b>13</b>	octanal	99.5		35.2	35.2
<b>16</b>	nonanal	99.9		13.2	26.4 <sup>h</sup>
<b>21</b>	decanal	99.8		17.6	17.6
<b>22</b>	linalool	99.7	49.7:50.3 <sup>f</sup>	148	74.1 <sup>h</sup>
<b>25</b>	4-terpineol	99.9+	78.5:21.5 <sup>f</sup>	702	702
<b>29</b>	isoborneol	98.7 <sup>b</sup>	49.1:50.9 <sup>f</sup>	15.8	15.8
<b>31</b>	$\alpha$ -terpineol	99.9	61.1:38.9 <sup>f</sup>	4,180	4,180
<b>32a</b>	<i>(-)</i> -borneol	99.3		99.3	99.3
<b>32b</b>	<i>(+)</i> -borneol	96.5 <sup>c</sup>		107	107
<b>36</b>	nerol	99.4		17.2	11.5 <sup>h</sup>
<b>39</b>	geraniol	99.8		23.3	23.3
<b>41</b>	guaiacol	99.8		1.5	1.5
<b>46</b>	methyleugenol	99.9+		8.7	8.7
<b>48</b>	<i>(E)</i> -cinnamaldehyde	99.9+		800	1,600 <sup>g</sup>
<b>51</b>	eugenol	99.9+		6.3	6.3
<b>53</b>	<i>(Z)</i> -isoeugenol	8.0 <sup>d</sup>		0.2	0.2
<b>54</b>	<i>(E)</i> -isoeugenol	92.0 <sup>d</sup>		2.7	2.7
<b>56</b>	coumarin	99.1		46.0	46.0
<b>58</b>	vanillin	99.9+		88.0	176 <sup>g</sup>

<sup>a</sup>Numbers correspond to those in **Tables 4.1, 4.2, 4.5, 4.6, Figure 4.2** and **Chapter 3**. <sup>b</sup>Contained additional 1.3% of borneol (**32**). <sup>c</sup>Contained additional 3.5% of isoborneol (**29**). <sup>d</sup>Mixture of two isomers. <sup>e</sup>Expressed as percentage. <sup>f</sup>Orders of enantiomers correspond to those in **Table 4.1**. <sup>g, h</sup>Adjusted by sensory or SPME data, respectively.

**Table 4.4** pH and chemical composition of de-carbonated cola.

	mean $\pm$ SD <sup>a</sup>	95% confidence interval
pH (at 25°C)	2.31 $\pm$ 0.03	2.24 – 2.39
soluble solids (°Brix, corrected to 20°C)	10.73 $\pm$ 0.06	10.59 – 10.87
titratable acidity (g of citric acid/100 mL)	0.1226 $\pm$ 0.0011	0.1198 – 0.1253
titratable acidity (g of phosphoric acid/100 mL)	0.0625 $\pm$ 0.0006	0.0610 – 0.0640

<sup>a</sup>Mean and standard deviation of products having three different code dates.

**Table 4.5** R-index values for omission test of group of compounds.

group <sup>a</sup>	odorants omitted <sup>b</sup>	R-index JB <sup>c</sup>
1 – citrus/floral	( <i>R</i> )-(+)-limonene ( <b>8b</b> ), linalool ( <b>22</b> ), nerol ( <b>36</b> ), geraniol ( <b>39</b> )	53.57
2 – herbal/cooling	1,8-cineol ( <b>9</b> ), 4-terpineol ( <b>25</b> ), isoborneol ( <b>29</b> ), $\alpha$ -terpineol ( <b>31</b> ), borneol ( <b>32a</b> and <b>32b</b> )	32.14
3 – aldehyde	octanal ( <b>13</b> ), nonanal ( <b>16</b> ), decanal ( <b>21</b> )	53.57
4 – vanilla	guaiacol ( <b>41</b> ), coumarin ( <b>56</b> ), vanillin ( <b>58</b> )	53.57
5 – brown spice	methyleugenol ( <b>46</b> ), ( <i>E</i> )-cinnamaldehyde ( <b>48</b> ), eugenol ( <b>51</b> ), ( <i>Z</i> )-isoeugenol ( <b>53</b> ), ( <i>E</i> )-isoeugenol ( <b>54</b> )	67.86 *

<sup>a</sup>Description of each group. <sup>b</sup>Compound numbers correspond to those in **Tables 4.1 – 4.3, 4.6, Figure 4.2** and **Chapter 3**. <sup>c</sup>Calculated by using John Brown computation against complete model and expressed as percentage. \*Significantly different from complete model at  $\alpha = 0.05$  (critical value for two-tailed test,  $\alpha = 0.05$ , and  $n = 28$  (19 females and 9 males; 20-48 years) is 17.65).

**Table 4.6** R-index values for omission studies of single compounds.

odorants omitted <sup>a</sup>	R-index JB <sup>b</sup>
methyleugenol ( <b>46</b> )	34.62
( <i>E</i> )-cinnamaldehyde ( <b>48</b> )	53.85
eugenol ( <b>51</b> )	50.00
( <i>Z</i> )- and ( <i>E</i> )-isoeugenols ( <b>53</b> and <b>54</b> )	42.31

<sup>a</sup>Compound numbers correspond to those in **Tables 4.1 – 4.3, 4.5, Figure 4.2** and **Chapter 3**. <sup>b</sup>Calculated by using John Brown computation against complete model and expressed as percentage (critical value for two-tailed test,  $\alpha = 0.05$ , and  $n = 26$  (18 females and 8 males; 20-48 years) is 18.25).

## VII. REFERENCES

- Abernethy, J.L. The historical and current interest in coumarin. *J. Chem. Educ.* **1969**, *46*, 561-568.
- ASTM Standard E679-04, 2004, Standard Practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits, ASTM International, West Conshohocken, PA, 2004, DOI: 10.1520/E0679-04, [www.astm.org](http://www.astm.org).
- Blank, I.; Fischer, K-H.; Grosch, W. Intensive neutral odourants of linden honey. *Z. Lebensm. Unters. Forsch.* **1989**, *189*, 426-433.
- Bugarčić, Z.M.; Dunkić, J.D.; Mojsilović, B.M. A simple, convenient and expeditious approach to cineol. *Heteroatom Chem.* **2004**, *15*, 468-470.
- Buttery, R.G.; Black, D.R.; Guadagni, D.G.; Ling, L.C.; Connolly, G.; Teranishi, R. California bay oil: I. Constituents, odor properties. *J. Agric. Food Chem.* **1974**, *22*, 773-777.
- Buttery, R.G.; Ling, L.C. Additional studies on flavor components of corn tortilla chips. *J. Agric. Food Chem.* **1998**, *46*, 2764-2769.
- Buttery, R.G.; Ling, L.C.; Light, D.M. Tomato leaf volatile aroma components. *J. Agric. Food Chem.* **1987**, *35*, 1039-1042.
- Buttery, R.G.; Takeoka, R.; Ling, L.C. Furanol: odor threshold and importance to tomato aroma. *J. Agric. Food Chem.* **1995**, *43*, 1638-1640.
- Buttery, R.G.; Teranishi, R.; Flath, R.A.; Ling, L.C. Fresh tomato volatiles, composition and sensory studies. In *Flavor Chemistry: Trends and Developments*; Teranishi, R., Buttery, R.G., Shahidi, F., Eds.; ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989; pp 213-222.
- Buttery, R.G.; Teranishi, R.; Ling, L.C.; Turnbaugh, J.G. Quantitative and sensory studies on tomato paste volatiles. *J. Agric. Food Chem.* **1990**, *38*, 336-340.
- Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006-1009.
- Ceruti, M.; Grosa, G.; Rocco, F.; Dosio, F.; Cattel, L. A convenient synthesis of [3-<sup>3</sup>H]squalene and [3-<sup>3</sup>H]-2,3-oxidosqualene. *J. Labelled Compd. Radiopharm.* **1994**, *34*, 577-585.
- Chen, H.; Chan, K.K.; Budd, T. Pharmacokinetics of *d*-limonene in the rat by GC-MS assay. *J. Pharm. Biomed. Anal.* **1998**, *17*, 631-640.

- Christlbauer, M.; Schieberle, P. Evaluation of the key aroma compounds in beef and pork vegetable gravies a la chef by stable isotope dilution assays and aroma recombination experiments. *J. Agric. Food Chem.* **2011**, *59*, 13122-13130.
- Clark, B.C., Jr.; Chamblee, T.S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, 1992; pp 229-285.
- Clos, J.F.; DuBois, G.E.; Prakash, I. Photostability of rebaudioside A and stevioside in beverages. *J. Agric. Food Chem.* **2008**, *56*, 8507-8513.
- Coppella, S.J.; Barton, P. Supercritical carbon dioxide extraction of lemon oil. In *Supercritical Fluids*; Squires, T.G., Paulaitis, M.E., Eds.; ACS Symposium Series 329; American Chemical Society: Washington, DC, 1987; pp 202-212.
- Corey, E.J.; Suggs, J.W. Pyridium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, *16*, 2647-2650.
- Czerny, M.; Mayer, F.; Grosch, W. Sensory study on the character impact odorants of roasted Arabica coffee. *J. Agric. Food Chem.* **1999**, *47*, 695-699.
- Dharmawan, J.; Kasapis, S.; Sriramula, P.; Lear, M.J.; Curran, P. Evaluation of aroma-active compounds in Pontianak orange peel oil (*Citrus nobilis* Lour. Var. *microcarpa* Hassk.) by gas chromatography-olfactometry, aroma reconstitution, and omission test. *J. Agric. Food Chem.* **2009**, *57*, 239-244.
- Durst, H.D.; Leete, E. Synthesis of geraniol-7-<sup>14</sup>C. *J. Labelled Compd.* **1971**, *7*, 52-55.
- Fischer, A; Grab, W.; Schieberle, P. Characterisation of the most odour-active compounds in a peel oil extract from Pontianak oranges (*Citrus nobilis* var. Lour. *microcarpa* Hassk.). *Eur. Food Res. Technol.* **2008**, *227*, 735-744.
- Furniss, B.S.; Hannaford, A.J.; Smith, P.W.G.; Tatchell, A.R. *Vogel's Textbook of Practical Organic Chemistry*, 5<sup>th</sup> edition; Longman Group UK: London, 1989; pp 530-531, 886-887, 1077-1079.
- Gil, A.; de la Fuente, E.B.; Lenardis, A.E.; Pereira, M.L.; Suarez, S.A.; Bandoni, A.; van Baren, C.; Lira, P.D.L.; Ghersa, C.M. Coriander essential oil composition from two genotypes grown in different environmental conditions. *J. Agric. Food Chem.* **2002**, *50*, 2870-2877.
- Gottfried, J.A.; Winston, J.S.; Dolan, R.J. Dissociable codes of odor quality and odorant structure in human piriform cortex. *Neuron* **2006**, *49*, 467-479.
- Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533-545.

- Guadagni, D.G.; Buttery, R.G.; Harris, J. Odour intensities of hop oil components. *J. Sci. Food Agric.* **1966**, *17*, 142-144.
- Guadagni, D.G.; Buttery, R.G.; Okano, S. Odour thresholds of some organic compounds associated with food flavours. *J. Sci. Food Agric.* **1963**, *14*, 761-765.
- Havens, B.R.; Meloan, C.E. The application of deuterated sex pheromone mimics of the American cockroach (*Periplaneta americana*, L.), to the study of Wright's vibrational theory of olfaction. In *Food Flavors: Generation, Analysis and Process Influence*; Charalambous, G., Ed.; Developments in Food Science 37A; Elsevier Science B.V.: Amsterdam, Netherlands, 1995; pp 497-524.
- Högnadóttir, Á.; Rouseff, R.L. Identification of aroma active compounds in orange essence oil using gas chromatography-olfactometry and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2003**, *998*, 201-211.
- House, K.A.; Acree, T.E. Sensory impact of free fatty acids on the aroma of a model cheddar cheese. *Food Qual. Prefer.* **2002**, *13*, 481-488.
- Huang, T-C.; Ho, C-T.; Fu, H-Y.; Pan, M-H. Cytotoxin properties of leaf essential oil and components from indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh). In *Herbs: Challenges in Chemistry and Biology*; Wang, M., Sang, S., Hwang, L.S., Ho, C-T., Eds.; ACS Symposium Series 925; American Chemical Society: Washington, DC, 2006; pp 299-313.
- Inukai, T.; Kojima, T. Catalytic actions of aluminum chloride on the isoprene-methyl acrylate diels-alder reaction. *J. Org. Chem.* **1966**, *31*, 1121-1123.
- Inukai, T.; Kojima, T. Diels-alder reactions of acrylic acid derivatives catalyzed by aluminum chloride. *J. Org. Chem.* **1965**, *30*, 3567-3569.
- Kerrola, K.; Kallio, H. Volatile compounds and odor characteristics of carbon dioxide extracts of coriander (*Coriandrum sativum* L.) fruits. *J. Agric. Food Chem.* **1993**, *41*, 785-790.
- Kotseridis, Y.; Baumes, R.; Skouroumounis, G.K. Synthesis of labeled [<sup>2</sup>H<sub>4</sub>]β-damascenone, [<sup>2</sup>H<sub>2</sub>]2-methoxy-3-isobutylpyrazine, [<sup>2</sup>H<sub>3</sub>]α-ionone, and [<sup>2</sup>H<sub>3</sub>]β-ionone, for quantification in grapes, juices and wines. *J. Chromatogr. A* **1998**, *824*, 71-78.
- Kulkarni, P.P.; Kadam, A.J.; Mane, R.B.; Desai, U.V.; Wadgaonkar, P.P. Demethylation of methyl aryl ethers using pyridine hydrochloride in solvent-free conditions under microwave irradiation. *J. Chem. Res., Synop.* **1999**, *6*, 394-395.
- Kurtz, A.J.; Lawless, H.T.; Acree, T.E. The cross-adaptation of green and citrus odorants. *Chem. Percept.* **2010**, *3*, 149-155.

- Laing, D.G. Perceptual odour interactions and objective mixture analysis. *Food Qual. Pref.* **1994**, *5*, 75-80.
- Laing, D.G.; Eddy, A.; Best, D.J. Perceptual characteristics of binary, trinary, and quaternary odor mixtures consisting of unpleasant constituents. *Physio. Behav.* **1994**, *56*, 81-93.
- Lin, J.; Welti, D.H.; Vera, F.A.; Fay, L.B.; Blank, I. Synthesis of deuterated volatile lipid degradation products to be used as internal standards in isotope dilution assays. 1. Aldehydes. *J. Agric. Food Chem.* **1999**, *47*, 2813-2821.
- Liu, F.; He, Y. Use of visible and near infrared spectroscopy and least squares-support vector machine to determine soluble solids content and pH of cola beverage. *J. Agric. Food Chem.* **2007**, *55*, 8883-8888.
- Liu, S-Q.; Holland, R.; Crow, V.L. Ester and their biosynthesis in fermented dairy products: a Review. *Int. Dairy J.* **2004**, *14*, 923-945.
- Lorjaroenphon, Y.; Cadwallader, K.R.; Kim, H.; Lee, S-Y. Evaluation of key odorants in chipotle pepper by quantitative analysis, calculation of odor activity values and omission studies. In *Recent Highlights in Flavor Chemistry and Biology*, Proceedings of 8<sup>th</sup> Wartburg Symposium on Flavor Chemistry & Biology, Eisenach, Germany, February 28–March 2, 2007; Hofmann, T., Meyerhof, W., Schieberle, P., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, 2008; pp 191-200.
- Masanetz, C.; Grosch, W. Hay-like off-flavour of dry parsley. *Z. Lebensm. Unters. Forsch. A* **1998a**, *206*, 114-120.
- Masanetz, C.; Grosch, W. Key odorants of parsley leaves (*Petroselinum crispum* [Mill.] Nym. Ssp. *crispum*) by odour-activity values. *Flavour Fragr. J.* **1998b**, *13*, 115-124.
- Meyer, S.D.; Schreiber, S.L. Acceleration of the Dess-Martin oxidation by water. *J. Org. Chem.* **1994**, *59*, 7549-7552.
- Minh Tu, N.T.; Thanh, L.X.; Une, A.; Ukeda, H.; Sawamura, M. Volatile constituents of Vietnamese pummel, orange, tangerine and lime peel oils. *Flavour Fragr. J.* **2002**, *17*, 169-174.
- Mistry, B.S.; Reineccius, T.; Olson, L.K. Gas chromatography-olfactometry for the determination of key odorants in foods. In *Techniques for Analyzing Food Aroma*; Marsili, R., Ed.; Marcel Dekker: New York, 1997; pp 265-292.
- Nicolaou, K.C.; Magolda, R.L.; Sipio, W.J.; Barnette, W.E.; Lysenko, Z.; Joullie, M.M. Phenylselenoetherification. A highly efficient cyclization process for the synthesis of *o*- and *s*-heterocycles. *J. Am. Chem. Soc.* **1980**, *102*, 3784-3793.

- Njoroge, S.M.; Ukeda, H.; Kusunose, H.; Sawamura, M. Volatile components of Japanese Yuzu and lemon oils. *Flavour Frag. J.* **1994**, *9*, 159-166.
- O'Mahony, M. Additional Nonparametric Tests. *Sensory Evaluation of Food: Statistical Methods and Procedures*; Marcel Dekker, Inc.: New York, 1986, pp 303-370.
- O'Mahony, M. Understanding discrimination tests: a user-friendly treatment of response bias, rating and ranking R-index tests and their relationship to signal detection. *J. Sensory Stud.* **1992**, *7*, 1-47.
- Ohloff, G. Importance of minor components in flavors and fragrances. *Perfum. Flavor.* **1978**, *3*, 11-22.
- Opdyke, D.L.J. Linalool. *Food Cosmet. Toxicol.* **1975**, *13*, 827-832.
- Padrayuttawat, A.; Yoshizawa, T.; Tamura, H.; Tokunaga, T. Optical isomers and odor thresholds of volatile constituents in *Citrus sudachi*. *Food Sci. Technol. Int. Tokyo* **1997**, *3*, 402-408.
- Pérez-Silva, A.; Odoux, E.; Brat, P.; Ribeyre, F.; Rodriguez-Jimenes, G.; Robles-Olvera, V.; García-Alvarado, M.A.; Günata, Z. GC-MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans. *Food Chem.* **2006**, *99*, 728-735.
- Pierce, J.D.; Wysocki, C.J., Jr.; Aronov, E.V.; Webb, J.B.; Boden, R.M. The role of perceptual and structural similarity in cross-adaptation. *Chem. Senses* **1996**, *21*, 223-237.
- Rabe, S.; Krings, U.; Berger, R.G. Dynamic flavor release from sucrose solutions. *J. Agric. Food Chem.* **2003**, *51*, 5058-5066.
- Ravi, R.; Prakash, M.; Bhat, K.K. Aroma characterization of coriander (*Coriandrum sativum* L.) oil samples. *Eur. Food Res. Technol.* **2007**, *225*, 367-374.
- Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München: Garching, Germany, 1998; p 47.
- Sawamura, M.; Tu, N.T.M.; Yu, X.; Xu, B. Volatile constituents of the peel oils of several sweet oranges in China. *J. Essent. Oil Res.* **2005**, *17*, 2-6.
- Scheidig, C.; Czerny, M.; Schieberle, P. Changes in key odorants of raw coffee beans during storage under defined conditions. *J. Agric. Food Chem.* **2007**, *55*, 5768-5775.

- Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
- Schneider, S.; Rolando, C. One step synthesis of vanillin  $d_3$  (4-hydroxy-3-(methoxy  $d_3$ )-benzaldehyde. *J. Labelled. Compd. Rad.* **1992**, *31*, 489-492.
- Schuh, C.; Schieberle, P. Characterization of the key aroma compounds in the beverages prepared from Darjeeling black tea: Quantitative differences between tea leaves and infusion. *J. Agric. Food Chem.* **2006**, *54*, 916-924.
- Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odourants in roasted coffee by stable isotope dilution assays. *Flavour Frag. J.* **1995**, *10*, 1-7.
- Senanayake, U.M.; Lee, T.H.; Wills, R.B.H. Volatile constituents of cinnamon (*Cinnamomum zeylanicum*) oils. *J. Agric. Food Chem.* **1978**, *26*, 822-824.
- Speed, T.J.; McIntyre, J.P.; Thamattoor, D.M. Wittig reaction using a stabilized phosphorus ylid: an efficient and stereoselective synthesis of ethyl trans-cinnamate. *J. Chem. Educ.* **2004**, *81*, 1355-1356.
- Steinhaus, M.; Fritsch, H.; Schieberle, P. Quantitation of (R)- and (S)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA). *J. Agric. Food Chem.* **2003**, *51*, 7100-7105.
- Steinhaus, M.; Schieberle, P. Characterization of odorants causing an atypical aroma in white pepper powder (*Piper nigrum* L.) based on quantitative measurements and orthonasal breakthrough thresholds. *J. Agric. Food Chem.* **2005**, *53*, 6049-6055.
- Toyoda, T.; Nohara, I.; Sato, T. Headspace analysis of volatile compounds emitted from various citrus blossoms. In *Bioactive Volatile Compounds from Plants*; Teranishi, R., Buttery, R.G., Sugisawa, H., Eds.; ACS Symposium Series 525; American Chemical Society: Washington, DC, 1993; pp 205-219.
- Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277-282.
- Watcharananun, W.; Cadwallader, K.R.; Huangrak, K.; Kim, H.; Lorjaroenphon, Y. Identification of predominant odorants in Thai desserts flavored by smoking with “Tian Op”, a traditional Thai scented candle. *J. Agric. Food Chem.* **2009**, *57*, 996-1005.
- Zhao, H.; Ivic, L.; Otaki, J.M.; Hashimoto, M.; Mikoshiba, K.; Firestein, S. Functional expression of a mammalian odorant receptor. *Science* **1998**, *279*, 237-242.

**CHAPTER 5**

**CHARACTERIZATION OF STORAGE-INDUCED CHANGES IN  
POTENT ODORANTS AND OTHER VOLATILE CONSTITUENTS OF  
COLA-FLAVORED CARBONATED BEVERAGES**

**I. ABSTRACT**

Aroma changes in cola-flavored carbonated beverages stored at various temperatures (5°C, room temperature, and 40°C) for 3 months were investigated by sensory and instrumental analysis. The result of R-index ranking revealed that aroma of cola stored at 40°C was significantly different from typical (fresh) cola. The aroma profiles by descriptive analysis also indicated that cola stored at room temperature and 40°C possessed a less intense lemon-lime note compared to typical cola and cola stored at 5°C. Furthermore, the caramel note of cola stored at 40°C was higher than that of other colas. The potent odorants of stored colas were identified by gas chromatography-olfactometry (GCO) and aroma extract dilution analysis (AEDA). The most potent odorants were eugenol and coumarin in all stored colas as well as in the initial sample (day 0). Octanal and linalool, known to be potent odorants in fresh cola, declined as a function of increasing storage temperature in the stored colas. Moreover, *p*-cresol was indicated as a potential off-flavor in storage-abused (40°C) cola. These results were in good agreement with the relative aroma concentrations determined by GC-mass spectrometry (GC-MS). Some key odorants of typical cola were not stable during storage due to temperature dependent acid-catalyzed reactions. In addition, 2-carene and two unidentified volatiles could be used as the chemical markers to indicate high temperature storage abuse.

## II. KEYWORDS

Cola-flavored carbonated beverage; acid-catalyzed reaction; storage-induced flavor; *p*-cresol; 2-carene

## III. INTRODUCTION

Cola-flavored carbonated beverages are responsible for 54.0% of the total global revenues for soft drinks and bottled water (IBISWorld, 2011). In 2010, cola in the United States accounted for about \$14 billion in sales (Kaczanowska, 2010a, 2010b). Despite its popularity, the flavor chemistry of cola is poorly understood. Cola flavor is mainly derived from the natural flavors listed on the commercial labels, and the flavorings may contain vanilla extract and essential oils of citrus, cassia or cinnamon, coriander, nutmeg, and neroli (Tchudi, 1986; Pendergrast, 2000). The mixture of these ingredients is responsible for cola's unique and complex flavor.

The volatile components of regular and diet colas have been identified (Wiley et al., 1984; Elmore et al., 1997; Hida et al., 1998), and the major aroma-active compounds in typical cola were determined to be terpenes and aldehydes (**Chapter 3**). Among the numerous aroma compounds in cola, the odorants making the greatest contribution were 1,8-cineol, linalool (both (*R*)-(-)- and (*S*)-(+)-enantiomers), octanal, nonanal, decanal, and (*R*)-(+)-limonene (**Chapter 4**). The results from omission studies also indicated that the characteristic aroma of cola was a result of a balance of numerous odorants. Omission of a mixture of methyleugenol, (*E*)-cinnamaldehyde, eugenol, and (*Z*)- and (*E*)-isoeugenols affected the overall aroma of cola model (**Chapter 4**).

*p*-Cymene has been proposed as an off-odor in cola stored at high temperature because of its odor quality (terpene note) and high abundance in an unusual sample (Wiley et al., 1984). They also reported that *p*-cymene was a degradation product of  $\gamma$ -terpinene and limonene from lemon oil by dehydrogenation reactions. On the other hand, several other compounds have been reported to cause off-flavors in lemon oil, including 2-*p*-tolyl-propene (Kimura et al., 1982), and *p*-cresol and *p*-methylacetophenone (Schieberle and Grosch, 1988).  $\alpha$ -*p*-Dimethylstyrene and *p*-cymen-8-ol were also reported as the off-flavors in carbonated citral-containing beverage (Peacock and Kuneman, 1985). These off-flavors may be more potent off-flavors than *p*-cymene in stored colas.

Not only formation of storage-induced flavors, but also deterioration of typical potent odorants may cause flavor changes of stored cola. Limonene can be converted to  $\alpha$ - and  $\beta$ -terpineols under acidic conditions, and the alcohols can undergo further transformation to (*Z*)- and (*E*)-1,8-terpins (Clark and Chamblee, 1992). Since limonene was present at highest abundance in colas (**Chapter 3**), loss of limonene by acid-catalyzed reactions may result in a different balance of odorants in the cola, resulting in flavor changes.

Thus, this study aimed to: 1) evaluate the potent odorants in colas stored at various temperatures by application of AEDA, 2) establish causes of aroma changes, including formation of storage-induced odorants and degradation of typical potent odorants by acid-catalyzed reactions, and 3) identify storage-induced volatiles which can be used as chemical markers to indicate storage temperature-abused cola. The results from this study could help in the development of technology to improve the stability and extend the shelf-life of cola-flavored carbonated beverages.

## IV. MATERIALS AND METHODS

### Materials

Samples of a commercial brand regular cola-flavored carbonated beverage and reference materials used in sensory descriptive analysis were obtained from local markets (Urbana, IL). The cola samples in original packaging (cans) were stored at 5°C, room temperature (~21.7°C), and 40°C for three months.

### Chemicals

Reagent or HPLC grade chemicals including anhydrous diethyl ether, anhydrous sodium sulfate, hydrochloric acid, methanol, sodium bicarbonate, and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ). Ultra high purity (UHP) nitrogen, and UHP helium were supplied by S.J. Smith (Davenport, IA).

*Reference standard compounds.* The standard compounds used to confirm the structure of the aroma compounds identified by GCO and GC-MS were obtained from the companies given in parentheses: compound nos. **1-6, 9-11, 13-18, 21, 22, 25, 27-31, 33, 34, 36, 38, 39, 42, 45, 46, 48, 49, 51, 52, 56, 60, 61, 63, 64, 67, 68, 79, 81, 96, 97, 106, and 108** (Sigma-Aldrich, St. Louis, MO); **19, 20, 23, 83, 86, 87, 90, 92, and 94** (Bedoukian, Danbury, CT); **24** (Firmenich, Princeton, NJ); **35 and 37** (Alfa Aesar, Lancashire, United Kingdom); **41 and 69** (Fluka, Buchs, Switzerland); **58** (TCI, Portland, OR); **101 and 105** (Nu-Chek Prep, Elysian, MN). 2,3-Dehydro-1,8-cineol (**53**) was synthesized according to the methodology described in **Chapter 3**.

## Sensory Analysis

The sensory analysis in this study was approved by The University of Illinois at Urbana-Champaign Institutional Review Board (IRB), and the human subject protocol number was 11249.

***R-index ranking test.*** The stored colas were determined the different from the typical cola using R-index ranking test followed the procedure in **Chapter 4**. The new lot of cola (typical cola; freshness by the expiration code date (~9 months shelf-life)) was used as the control. All cola samples (50 mL) were prepared in 125-mL Teflon sniff bottles (Nalgene PTFE wash bottle without siphon tube; Nalge Nunc International, Rochester, NY) covered with aluminum foil to avoid assessor bias. The colas were naturally de-carbonated in the bottle 30 min before testing. A set of test samples, including stored cola at various temperatures and typical cola, were labeled with random 3-digit codes, and presented to the panelists with control sample. The serving order was random and balance. Twenty three students and staff (16 females and 7 males; 18-61 years) participated in this test. They were asked to sniff the expressed air by gently squeezing the bottle, and ranked the test samples on how similar they were to the control sample (typical cola). John Brown computation as described by O'Mahony (1992) was used to calculate the R-index which is the percentage of times the sample is ranked less similar to the control sample. The percentage of R-index was compared to the critical value ( $n = 23$ ) for two-tailed test at  $\alpha = 0.05$  to detect the significant differences from the typical cola.

***Descriptive analysis.*** The aromas by nose of stored colas were created and evaluated by ten panelists (university students and staff; 7 females and 3 males; 22-45 years). They were selected and trained (15 h for new panelists and 3 h of refresher training for panelist who had previously participated in cola evaluation in **Chapter 3**) to rate the intensity of aromas on 15-cm

line with two end anchors (none and strong) as describe in **Chapter 3**. The stored colas and typical cola were prepared in the sniff bottles as mentioned above. The trained panelists evaluated the samples (one by one) at room temperature in individual booths. The analysis of variance (ANOVA) of each attribute was performed using the SAS<sup>®</sup> program (SAS institute Inc., Cary, NC) to detect significant differences among cola samples. If there was a significant difference ( $p$ -value < 0.05), the least significant difference (LSD) was tested to check which samples differed from one another. The means of aromas by nose were plotted to establish the aroma profile as spider web plots.

### **Characterization of Potent Odorants**

*Isolation of aroma compounds.* Volatile compounds of stored colas were isolated using continuous liquid-liquid extraction (CLLE) as the detailed in **Chapter 3**. One can of initial or stored cola was extracted by diethyl ether in CLLE apparatus (part no. Z562440; Sigma-Aldrich) for 18 h. The aroma compounds were isolated from any non-volatile compounds by solvent assisted flavor evaporation (SAFE) (Engel et al., 1999) following the procedure of Watcharananun et al. (2009). The aroma extract in diethyl ether layer was washed with aqueous 0.5 M NaHCO<sub>3</sub> (3 × 20 mL) to obtain the neutral-basic fraction in organic layer. The aqueous layer was acidified to pH 2 with aqueous 4 M HCl, and then extracted with diethyl ether (3 × 20 mL). The solvent layer was combined as the acidic fraction. Each fraction was washed with saturated aqueous NaCl (2 × 15 mL), and concentrated to 10 mL by Vigreux column distillation at 43°C. The extract was dried over 2 g of anhydrous Na<sub>2</sub>SO<sub>4</sub>, and further concentrated to 350 μL before analysis.

**Identification of aroma-active compounds.** The aroma compounds were identified by gas chromatography-olfactometry (GCO) and GC-mass spectrometry (GC-MS) as described in **Chapter 3**.

**GCO.** The aroma extract (2  $\mu$ L) was injected in the cool on-column mode (+3°C oven tracking mode) into a 6890 GC (Agilent Technologies, Inc., Santa Clara, CA) equipped with a flame ionization detector (FID) and olfactory detection port (DATU Technology Transfer, Geneva, NY). Separations were performed on RTX<sup>®</sup>-Wax and RTX<sup>®</sup>-5 SILMS capillary columns (both 15 m  $\times$  0.32 mm i.d.  $\times$  0.5  $\mu$ m df; Restek, Bellefonte, PA). The column effluent was split 1:5 between the FID (250°C) and olfactory detection port (250°C), respectively. The oven temperature was programmed from 40°C to 225°C at a ramp rate of 10°C/min for the RTX<sup>®</sup>-Wax column or a ramp rate of 6°C/min for the RTX<sup>®</sup>-5 SILMS column, with initial and final hold times of 5 and 20 min, respectively. The flow rate of the helium carrier gas was 2 mL/min.

**GC-MS.** The GC-MS system consisted of a 6890 GC/5973 mass selective detector (Agilent Technologies, Inc.). One microliter of extract was injected into either the Stabilwax<sup>®</sup> (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m df; Restek) and SAC<sup>TM</sup>-5 (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m df; Supelco, Bellefonte, PA) capillary columns in the cool on-column mode. The initial oven temperature was 35°C. After 5 min, the oven temperature was increased at 4°C/min to a final temperature (225°C for Stabilwax<sup>®</sup> or 240°C for SAC<sup>TM</sup>-5), and held for 20 min. The flow rate of helium carrier gas was 1 mL/min. The mass spectra were performed in full scan mode (35-300 a.m.u., scan rate 5.27 scans/s, interface temperature 280°C, and ionization energy 70 eV).

The retention index (RI) of each compound was calculated against a series of standard *n*-alkanes (van den Dool and Kratz, 1963). The volatiles were positive identified based on

comparison of their RI values on two different polarity stationary phases of capillary columns and mass spectra against those of authentic standard compounds. Tentative compound identifications were based on comparison of their mass spectra against those in the NIST2008 mass spectral database when the authentic standards were not available.

***Aroma extract dilution analysis.*** This technique (AEDA) was conducted as previously described in **Chapter 3**. The extract was diluted stepwise in 1:3 (v/v) dilutions using diethyl ether. Each dilution was performed by GCO on RTX<sup>®</sup>-Wax column. The highest dilution at which panelists could smell a specific aroma compound was recorded as the flavor dilution (FD) factor for that compound. The FD values in this experiment were derived from average  $\log_3$ FD factors (n = 3; 2 females and 1 male; 21-38 years) after rounding off to the nearest whole number.

### **Investigation of Storage-Induced Volatiles**

***Isolation and separation of volatile compounds.*** Diethyl ether (50 mL) was placed in 250-mL centrifuge Teflon bottle before adding the cola sample to avoid loss of volatiles. Then, about 100 mL of cola was poured from the original can into the bottle. The exact weight of cola was recorded as weight difference. *tert*-Butylbenzene and 2-ethylbutanoic acid in methanol, used as internal standard, were added into the cola layer (each 50 ng/g cola sample). The sample was de-carbonated by sonication (model 2200; Branson Ultrasonics, Co., Danbury, CT) using an ice-water bath at 10°C for 30 min. Sodium chloride (20 g) was added into the sample before extraction at 200 rpm using DS-500 orbital shaker (VWR Scientific, Batavia, IL). After 30 min, the sample was centrifuged at 4,000 rpm (IEC HN-SII centrifuge; International Equipment Co., Needham Heights, MA) for 5 min to break the emulsion. The aroma extract was fractionated

into acidic and neutral-basic fractions, and then concentrated to 200  $\mu\text{L}$  in the same manner as described above. The GC-MS parameters for Stabilwax<sup>®</sup> and SAC<sup>™</sup>-5 capillary columns were the same as previously described.

***Identification and semi-quantification.*** The criteria for identification of aroma-active compounds were included use of RIs on two columns with different polarity stationary phases, odor properties determined by GCO, and mass spectrum against commercially available standard compounds. MSD ChemStation software (Agilent Technologies, Inc.) was used to integrate the area of total ions of each compound, and the mass of target volatile was calculated against internal standard compound using response factor ( $R_f$ ) = 1. The concentration was reported as ng/g of cola sample (n = 4), and the statistical analysis was conducted as describe in sensory descriptive analysis part.

## **V. RESULTS AND DISCUSSION**

The temperatures used to investigate flavor changes of stored colas for three months were 5°C, room temperature, and 40°C. The average ambient temperature was  $21.7 \pm 2.0^\circ\text{C}$ . The temperature at 40°C was used to accelerate the degradation, and it could be considered to represent a worst-case scenario for warehouse storage.

### **Sensory Changes**

R-index ranking test was used to verify if colas stored at various temperatures for three months had flavor changes. The results revealed that the overall aroma of cola stored at 40°C was significantly different from that of typical cola, while the aroma of colas stored at 5°C and room temperature were similar to that of the fresh sample (**Table 5.1**). Wiley et al. (1984) stated

that cola stored at 20°C had a terpene-like odor at the end of their shelf-life, but it should be noted that the cola sample used in the present study was very fresh (about nine months before the expiration date). So, changes in the aroma of cola stored at room temperature for three months may not be detected.

Aroma profiles of stored colas obtained from sensory descriptive analysis are shown in **Figure 5.1 (Table D.4)**. The aroma profile of cola stored at 5°C was similar to that of typical cola, while room temperature and 40°C stored colas had statistically lower intensities of the lemon-lime note. In addition, the caramel attribute of cola stored at 40°C was higher than that of other samples. Pine note of this cola was also higher compared to the other treatments, but it was not statistically different.

### **Aroma-Active Compounds**

Among several aroma-active compounds, eugenol (**33**) and coumarin (**38**) were the most potent odorants in all colas, including initial sample (0 day), colas stored at 5°C, at room temperature, and at 40°C (**Table 5.2** and the log<sub>3</sub> flavor dilution (FD) chromatograms are shown in **Figure B.2**). Previously, these two aroma components were the most potent odorants in the top three US brands of colas (**Chapter 3**). The results of aroma extract dilution analysis (AEDA) indicated that no additional potent odorants were formed in any stored colas, but the potency of some typical odorants decreased. FD factor of linalool (**11**) in cola stored at 40°C dramatically decreased compared to its value in the initial sample. Moreover, decanal (**10**), linalool (**11**), and (*E*)-isoeugenol (**37**) were not detected in the cola stored at 40°C (**Table 5.3**). Loss of these compounds might allow other odorants with pine and caramel notes to be more easily detected in storage-abused cola. In addition, the quantification data indicated a dramatic

loss of aroma compounds found in high abundance in typical colas, such as *d*-limonene (**2**) and  $\alpha$ -terpineol (**17**). Decrease of major components could result in changes in the partitioning of aroma compounds above the headspace, which in turn could affect the overall aroma of this product.

Losses of these typical potent odorants were most likely due to acid-catalyzed reactions. The degradation pathways of limonene and linalool were reviewed by Clark and Chamblee (1992). Limonene (**2**) is hydrated to  $\alpha$ - and  $\beta$ -terpineols (**17**, **77**, and **85**), and then hydrated to (*Z*)- and (*E*)-1,8-terpins (**112** and **109**). Another secondary product is 1,8-cineol (**3**) which can be derived from 1,8-terpins by dehydration reactions at high temperature. This resulted in a large increase in 1,8-cineol in cola stored at 40°C (**Table 5.3**). In addition, limonene can rearrange to form terpinolene (**58**),  $\gamma$ -terpinene (**56**), and  $\alpha$ -terpinene (**1**).

Under acidic conditions, linalool (**11**) is cyclized to  $\alpha$ -terpineol (**17**). Additionally, linalool can be converted to nerol (**20**) and geraniol (**23**), and both of these isomers can also rearrange to linalool. However, the reaction rate of conversion of linalool to geraniol and nerol is slower (Cori, 1986), which might explain why nerol and geraniol were not detected in cola stored at room temperature. The conversion rate of geraniol and nerol to linalool and  $\alpha$ -terpineol is affected by pH (Ohta et al., 1991), but the reactions of these citral alcohols do not depend on type of acid (Baxter et al., 1978). The secondary product,  $\alpha$ -terpineol, of these three alcohols further deteriorated under acidic environment as previously described.

The stability of typical potent odorants in cola based on relative concentrations is summarized in **Table 5.4**. Most of them were not stable during storage because of acid-catalyzed reactions. Additionally, degradations of *d*-limonene (**2**), octanal (**5**), nonanal (**6**), decanal (**10**), linalool (**11**), and eugenol (**33**) were temperature dependent. On the other hand,

deterioration rates for guaiacol (**25**), (*E*)-cinnamaldehyde (**30**), and vanillin (**41**) were independent of storage temperature. Among the potent odorants of typical cola, coumarin (**38**) was stable during storage because of its aromatic chemical structure and coumarin contains a five-carbon (lactone) atom ring which has high stability according to the “Spannungs-Theorie” of Baeyer (Dodge, 1916).

In comparison to the initial sample, some aroma-active compounds became more important in colas stored at ambient temperature and/or 40°C, for example butanoic acid (**15**) and, in particular, *p*-cresol (**31**) which had higher odor potencies in storage-abused cola (**Table 5.2, Figure B.2**). Increase in potency of butanoic acid in stored cola was in agreement with the increase in its relative concentration (**Table 5.3**). Unfortunately, the relative concentration of *p*-cresol could not be determined because it was present at only a trace concentration.

*p*-Cresol is reported as one of the most potent odorant in stored lemon oil (Schieberle and Grosch, 1988, 1989). Increase potency of *p*-cresol was, however, unexpected in cola-flavored carbonated beverage since it is the oxidation product from citral under acidic condition (Schieberle et al., 1988; Ueno et al., 2004, 2005; Ueno et al., 2006). Oxygen content of carbonated soft drinks does not exceed 0.5 ppm (Steen, 2006), and some of the aroma compounds found in cola have antioxidant properties, for example eugenol in clove oil (Jirovetz et al., 2006). This might explain the loss of eugenol (**33**) and its derivatives (**29** and **37**), which have antioxidant properties (**Table 5.3**). Therefore, it is possible that formation of *p*-cresol was due to oxidation from trace amounts of oxygen in the product. Moreover, increase of storage temperature can increase rate of oxidation reactions. The reaction rate of terpene deterioration may increase by 2-3 fold when temperature is increased by 10-15°C (Clark and Chamblee, 1992). Another possible reason was due to the second theory of oxidation which consists of two

steps including linking of water molecule at double bond and then hydrogen transfer to other reducible component (Shillinglaw and Levine, 1943). Ueno et al. (2006) stated, however, that formation of *p*-cresol from citral requires the direct reaction with an oxygen molecule. Formation mechanism of *p*-cresol was proposed by their team (Ueno et al., 2004, 2005; Ueno et al., 2006). *p*-Mentha-1,5-dien-8-ol (which is the intermediate of citral degradation) dehydrates to *p*-mentha-1,4(8),5-triene, and then oxidized to *p*-cresol.

On the other hand, *p*-cymene which was previously implicated as an off-odor in cola (Wiley et al., 1984) was not a potent odorant in stored colas. It was not reported earlier as the aroma-active compound from citral degradation (Schieberle et al., 1988).

### **Storage-Induced Changes in Volatile Composition**

Acid-catalyzed reactions not only result in degradation of typical potent odorants, but also cause the formation of storage-induced volatiles. The volatile profiles of stored colas were different from the initial sample, especially cola stored at 40°C (**Figure A.2**). The most abundant volatile compound in 40°C treatment was (*E*)-1,8-terpin (**109**), while *d*-limonene (**2**) was the major aroma of initial sample and cola stored at 5°C (**Tables 5.3** and **5.5**). Increase of (*E*)-1,8-terpin in stored colas was also in good agreement with a decrease of *d*-limonene by acid-catalyzed reactions as recently described.

$\alpha$ -Terpineol (**17**) which was the second most abundant compound in typical colas (**Chapters 3** and **4**) was present at highest concentration in cola stored at room temperature. Its relative abundance gradually increased during storage at ambient temperature because of formation from several volatiles and also its conversion to other secondary products as mentioned above. In addition, the accumulation of  $\alpha$ -terpineol over  $\beta$ -terpineols (**77** and **85**) was

due to the double bond position of limonene structure. The hydration rate of an exocyclic double bond is about 10 times faster than that of an endocyclic double bond (Clark and Chamblee, 1992). Furthermore, decrease of  $\alpha$ -terpineol in cola stored at 40°C correlated well with increase of the secondary product, 1,8-cineol (**3**).

Apart from *p*-cresol, there were many volatiles in colas derived via citral (mixture of neral and geranial) degradation, so it is important to discuss deterioration of citral even if it was not detected in any cola because the half-life of citral in carbonated soft drink is only six days (Peacock and Kuneman, 1985). The degradation of citral under acidic condition has been studied (Clark et al., 1977; Kimura et al., 1982; Kimura et al., 1983a, 1983b; Peacock and Kuneman, 1985; Schieberle et al., 1988; Grein et al., 1994; Ueno et al., 2004, 2005; Ueno et al., 2006), and the degradation mechanism in carbonated beverage model has been proposed (Lorjaroenphon et al., 2012). Geranial and neral can isomerize to each other, and neral converts to a carbonium ion by cyclization reaction. The latter component is converted to (*Z*)- and (*E*)-isopiperitenols, which then rearrange to (*Z*)- and (*E*)-*p*-mentha-2,8-dien-1-ols.

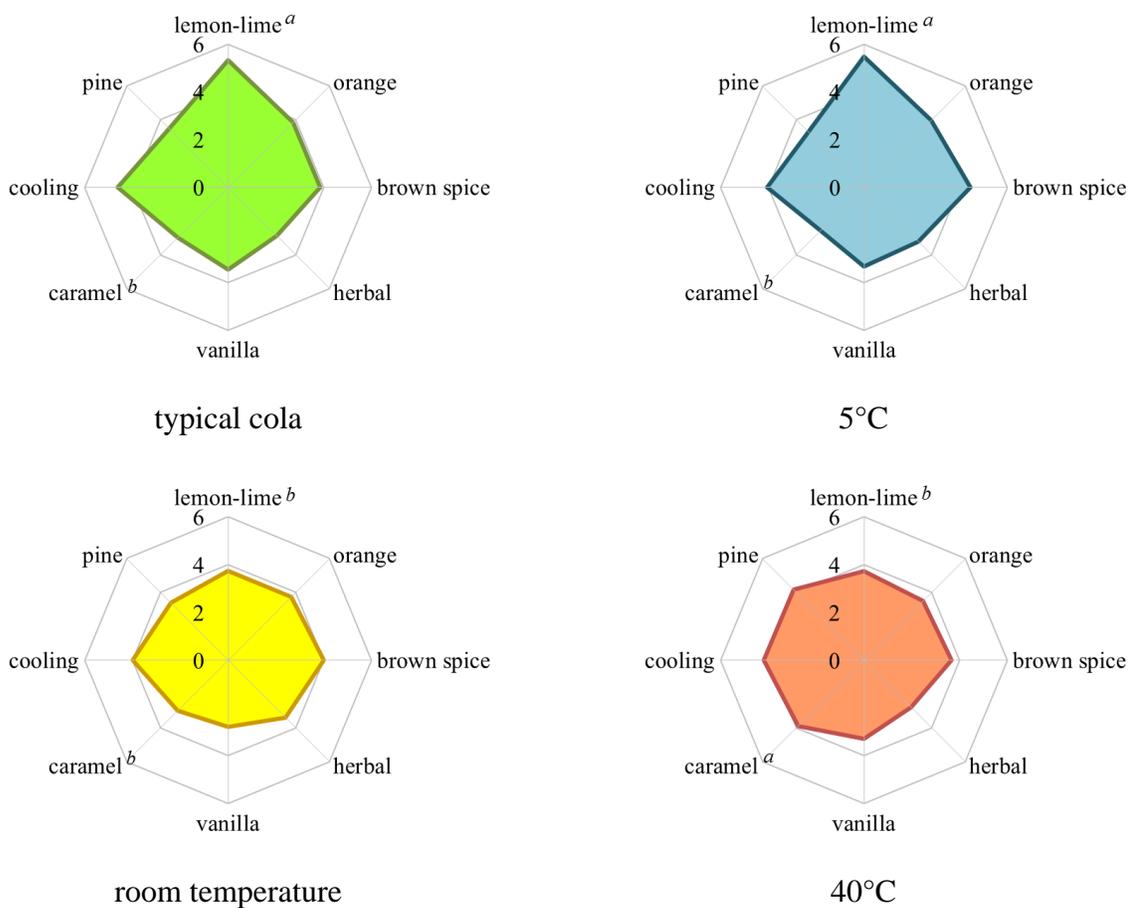
The carbonium ion from cyclization of neral can also hydrate to (*Z*)- and (*E*)-*p*-menth-1-ene-3,8-diols. These diols not only transform to *p*-mentha-1(7),2-dien-8-ol (**98**) and *p*-mentha-1,5-dien-8-ol (**88**) by dehydration, but also rearrange to (*Z*)- and (*E*)-*p*-menth-2-ene-1,8-diols (**113** and **110**). These four compounds rearrange to one another by hydration, dehydration, or isomerization reactions, but only *p*-mentha-1,5-dien-8-ol (**88**) and (*Z*)-*p*-menth-2-ene-1,8-diol (**113**) can form 2,3-dehydro-1,8-cineol (**53**). In addition, *p*-mentha-1,5-dien-8-ol (**88**) transforms to more stable products including *p*-cymene (**4**) by dehydration and *p*-cymen-8-ol (**100**) by disproportionation and redox reactions. The latter volatile is further dehydrated to  $\alpha$ -*p*-dimethylstyrene (**7**; also named dehydro-*p*-cymene).

*p*-Methylacetophenone (**96**) was proposed to be a product of oxidation reaction from  $\alpha$ -*p*-dimethylstyrene (Schieberle and Grosch, 1988; Schieberle et al., 1988; Ueno et al., 2004). It may also be derived from *p*-mentha-1,4(8),5-triene by oxidation (Ueno et al., 2005; Ueno et al., 2006). In addition, direct reaction with an oxygen molecule is required to form *p*-methyl acetophenone as well as *p*-cresol (Ueno et al., 2006). Presence of *p*-methylacetophenone in colas stored at room temperature and 40°C (**Table 5.5**) confirmed that oxidation occurs in cola-flavored carbonated beverages. The result of this present study also indicated that oxidation rate was temperature dependent. *p*-Methylacetophenone is reported as one of the potent odorant in stored lemon oil (Schieberle and Grosch, 1988, 1989). It was not, however, regarded as an off-odor in stored colas because of its higher odor detection threshold as compared to *p*-cresol. The threshold in air of *p*-methylacetophenone (2.7 - 10.8 ng/L) is about 10 times greater than that of *p*-cresol (0.3 - 1.0 ng/L) (Schieberle and Grosch, 1988).

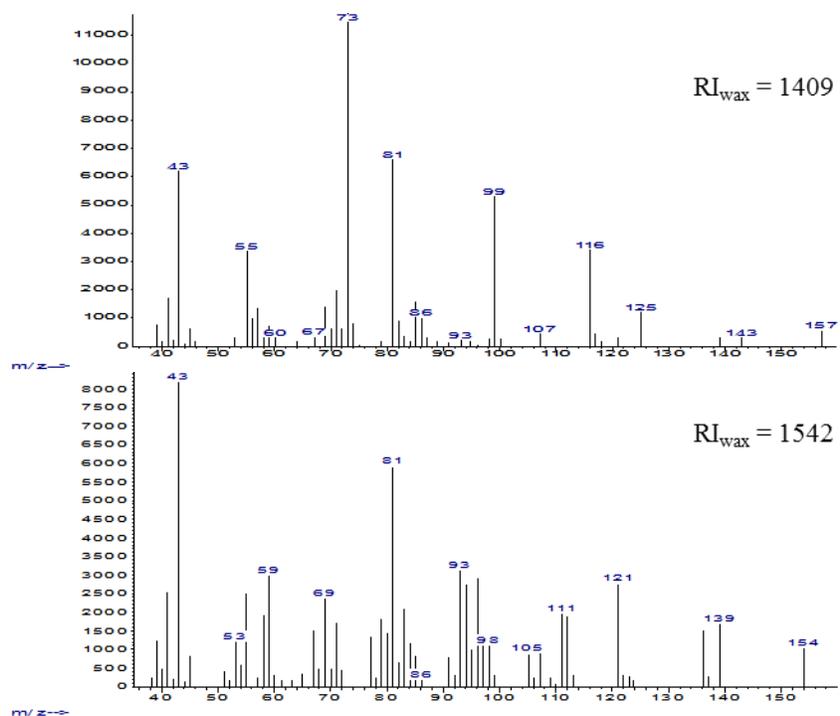
Furfural (**64**) and 5-hydroxymethylfurfural (**121**), which are degradation products of acids and sugars (Shaw et al., 1967; Lee and Nagy, 1996; Lo et al., 2008), also increased after storage (**Table 5.5**). Furfural is reported as a marker compound to indicate storage abuse of processed orange juice (Nagy and Randall, 1973), but in the present study it was present in all stored cola samples. Comparison among 121 volatiles found in stored colas investigated that there are three compounds which were present only in storage-abused cola. They were 2-carene (**48**) and two unidentified volatiles (**62** and **70**). The mass spectra of latter two components are shown in **Figure 5.2**. It should be, however, noted that these three unique volatiles are present at relatively low concentrations. 2-Carene is reported as an uncommon volatile compound in tomato leaf (Buttery et al., 1987), and it may form by isomerization of  $\delta$ -3-carene (**49**).

In summary, changes of cola aroma during storage were due to both degradation of typical potent odorants and formation of storage-induced aromas. *p*-Cresol was, for the first time, identified as off-odor compound in storage-abused cola. Investigation of this compound could be used to control the quality of the product, and at least one additional volatile compound could also be used as a chemical marker to indicate temperature abuse during storage.

## VI. FIGURES AND TABLES



**Figure 5.1** Aroma (by nose) profiles of colas stored at various temperatures for three months. Attributes followed by different letters are significantly different ( $p$ -value  $< 0.05$ ). Average intensities correspond to those in **Table D.4** ( $n = 10$ ; 7 females and 3 males; 22-45 years).



**Figure 5.2** Electron-impact mass spectra of unidentified chemical markers in storage-abused cola.

**Table 5.1** R-index values of colas stored at various temperatures for three months.

stored cola	R-index JB <sup>a</sup>
5°C	56.52
room temperature	60.87
40°C	69.57 *

<sup>a</sup>Calculated by using John Brown computation against typical cola and expressed as percentage. \*Significantly different from typical cola at  $\alpha = 0.05$  (critical value for two-tailed test,  $\alpha = 0.05$ , and  $n = 23$  (16 females and 7 males; 18-61 years old) is 19.28).

**Table 5.2** Potent odorants (FD  $\geq$  9) in colas stored at various temperatures for three months.

no. <sup>a</sup>	RI <sup>b</sup>		F <sup>c</sup>	compound	odor <sup>d</sup>	FD factor <sup>e</sup>			
	wax	RTX-5				initial	5°C	room temp.	40°C
3	1203	1042	NB	1,8-cineol	fresh, camphorous	27	27	27	81
5	1287	1002	NB	octanal	orange, citrus, green	27	9	9	3
6	1393	1103	NB	nonanal	green, lemon, floral	9	9	3	3
9	1434	< 700	A	acetic acid	vinegar	9	27	9	27
11	1542	1108	NB	linalool	floral, sweet	243	243	27	9
14	1603	1197	NB	4-terpineol	earthy, woody	27	27	27	3
15	1611	807	A	butanoic acid	cheesy, stinky	3	9	27	27
16	1663	1175	NB	isoborneol	earthy, camphorous	27	27	27	81
18	1702	1190	NB	borneol	camphorous, earthy	9	9	9	81
20	1770	1231	NB	nerol	sweet, floral	27	27	3	3
21	1804	1327	NB	( <i>E,E</i> )-2,4-decadienal	oily, fatty	3	9	< 3	3
24	1815	1385	NB	$\beta$ -damascenone	apple sauce, honey	9	9	9	9
25	1841	1089	NB	guaiacol	smoky	243	81	81	81
28	2010	1064	NB	Furaneol <sup>TM</sup>	burnt sugar	243	243	81	243
30	2024	1287	NB	( <i>E</i> )-cinnamaldehyde	cinnamon, sweet	27	27	27	27
31	2062	1093	NB	<i>p</i> -cresol	dung, fecal	3	< 3	< 3	81
33	2146	1356	NB	eugenol	clove, spicy, basil	729	729	729	729
34	2168	1128	A	sotolon	curry, burnt sugar, soy sauce	81	81	27	81
35	2244	1420	NB	( <i>Z</i> )-isoeugenol	spicy, basil	729	243	243	243
37	2332	1451	NB	( <i>E</i> )-isoeugenol	sweet, spicy	27	3	9	3
38	2419	1454	NB	coumarin	sweet, herbaceous	729	729	729	2,187
39	2525	1263	A	phenylacetic acid	rosy, floral	27	n.d. <sup>f</sup>	n.d. <sup>f</sup>	n.d. <sup>f</sup>
41	2531	1403	A	vanillin	vanilla	243	243	243	243

<sup>a</sup>Number correspond to those in **Tables 5.3** and **5.4**. <sup>b</sup>Retention indices determined by GCO on two different stationary phases (RTX<sup>®</sup>-Wax and RTX<sup>®</sup>-5 SILMS). <sup>c</sup>Fraction: acidic (A); neutral-basic (NB). <sup>d</sup>Odor quality determined by GCO. <sup>e</sup>Flavor dilution factors determined on RTX<sup>®</sup>-Wax column (n = 3; 2 females and 1 male; 21-38 years). <sup>f</sup>Not detected.

**Table 5.3** Relative concentration of selected aroma-active compounds in colas stored at various temperatures for three months.

no. <sup>a</sup>	RI <sup>b</sup>			compound	identification <sup>g</sup>	relative concentration (ng/g; ppb) <sup>h</sup>				p-value
	wax	SAC-5	F <sup>e</sup>			initial	5°C	room temp.	40°C	
1	1174	1013	NB	$\alpha$ -terpinene	MS, RI, S, O	206 ( $\pm$ 3%)a	177 ( $\pm$ 4%)b	102 ( $\pm$ 3%)c	78.9 ( $\pm$ 5%)d	<0.0001
2	1197	1031	NB	<i>d</i> -limonene	MS, RI, S, O	4,810 ( $\pm$ 8%)a	2,960 ( $\pm$ 6%)b	558 ( $\pm$ 3%)c	14.5 ( $\pm$ 9%)d	<0.0001
3	1201	1025	NB	1,8-cineol	MS, RI, S, O	57.4 ( $\pm$ 11%)c	50.3 ( $\pm$ 6%)d	91.7 ( $\pm$ 2%)b	193 ( $\pm$ 2%)a	<0.0001
4	1267	1020	NB	<i>p</i> -cymene	MS, RI, S, O	96.4 ( $\pm$ 2%)c	107 ( $\pm$ 1%)b	118 ( $\pm$ 1%)a	76.7 ( $\pm$ 3%)d	<0.0001
5	1289	1000	NB	octanal	MS, RI, S, O	11.7 ( $\pm$ 5%)a	9.7 ( $\pm$ 1%)b	7.1 ( $\pm$ 1%)c	5.9 ( $\pm$ 4%)d	<0.0001
6	1393	1101	NB	nonanal	MS, RI, S, O	17.0 ( $\pm$ 2%)a	14.6 ( $\pm$ 5%)b	9.4 ( $\pm$ 2%)c	6.2 ( $\pm$ 9%)d	<0.0001
7	1435	n.d. <sup>d</sup>	NB	$\alpha$ - <i>p</i> -dimethylstyrene	MS, O	2.2 ( $\pm$ 5%)b	0.9 ( $\pm$ 4%)d	1.2 ( $\pm$ 5%)c	2.5 ( $\pm$ 6%)a	<0.0001
8	1438	1069	NB	( <i>Z</i> )- or ( <i>E</i> )-linalool oxide	MS, RI, O	0.8 ( $\pm$ 5%)c	1.0 ( $\pm$ 10%)ab	0.9 ( $\pm$ 6%)b	1.1 ( $\pm$ 10%)a	0.0002
9	1467	n.d. <sup>d</sup>	A	acetic acid	MS, S, O	119 ( $\pm$ 41%)	129 ( $\pm$ 21%)	106 ( $\pm$ 5%)	122 ( $\pm$ 25%)	0.7688
10	1499	1207	NB	decanal	MS, RI, S, O	13.2 ( $\pm$ 13%)a	7.5 ( $\pm$ 7%)b	3.7 ( $\pm$ 12%)c	-	<0.0001
11	1550	1101	NB	linalool	MS, RI, S, O	120 ( $\pm$ 5%)a	82.3 ( $\pm$ 4%)b	8.3 ( $\pm$ 3%)c	-	<0.0001
12	1577	1133	NB	1-terpinenol <sup>f</sup>	MS, RI, O	53.7 ( $\pm$ 6%)c	60.5 ( $\pm$ 6%)c	179 ( $\pm$ 3%)b	375 ( $\pm$ 17%)a	<0.0001
13	1590	1414	NB	$\beta$ -caryophyllene	MS, RI, S, O	48.1 ( $\pm$ 14%)	51.4 ( $\pm$ 15%)	-	-	0.5521
14	1600	1177	NB	4-terpineol	MS, RI, S, O	593 ( $\pm$ 6%)a	419 ( $\pm$ 5%)b	376 ( $\pm$ 3%)c	75.7 ( $\pm$ 11%)d	<0.0001
15	1649	806	A	butanoic acid	MS, RI, S, O	7.5 ( $\pm$ 13%)c	10.0 ( $\pm$ 18%)c	29.9 ( $\pm$ 4%)b	45.0 ( $\pm$ 14%)a	<0.0001
16	1664	1153	NB	isoborneol	MS, RI, S, O	19.3 ( $\pm$ 6%)c	19.4 ( $\pm$ 4%)c	81.9 ( $\pm$ 3%)a	71.7 ( $\pm$ 15%)b	<0.0001
17	1703	1203	NB	$\alpha$ -terpineol <sup>f</sup>	MS, RI, S, O	2,990 ( $\pm$ 7%)b	2,630 ( $\pm$ 4%)c	3,230 ( $\pm$ 5%)a	413 ( $\pm$ 12%)d	<0.0001
18	1704	1164	NB	borneol <sup>f</sup>	MS, RI, S, O	230 ( $\pm$ 6%)a	164 ( $\pm$ 6%)b	171 ( $\pm$ 5%)b	114 ( $\pm$ 18%)c	<0.0001
19	1727	1364	NB	nerylacetate	MS, RI, S, O	110 ( $\pm$ 15%)a	56.4 ( $\pm$ 13%)b	-	-	0.0011
20	1801	1244	NB	nerol	MS, RI, S, O	13.7 ( $\pm$ 10%)	13.9 ( $\pm$ 6%)	-	-	0.8346
21	1812	n.d. <sup>d</sup>	NB	( <i>E,E</i> )-2,4-decadienal	MS, S, O	1.2 ( $\pm$ 14%)	-	-	-	-
22	1815	1254	NB	2-phenylethylacetate	MS, RI, S, O	5.8 ( $\pm$ 6%)	5.9 ( $\pm$ 6%)	-	-	0.5847
23	1851	1248	NB	geraniol	MS, RI, S, O	17.4 ( $\pm$ 19%)a	12.3 ( $\pm$ 6%)b	-	-	0.0219

**Table 5.3** (continued)

no. <sup>a</sup>	RI <sup>b</sup>		F <sup>c</sup>	compound	identification <sup>g</sup>	relative concentration (ng/g; ppb) <sup>h</sup>				p-value
	wax	SAC-5				initial	5°C	room temp.	40°C	
24	1815 <sup>c</sup>	1385 <sup>c</sup>	NB	β-damascenone	RI, S, O	-	-	-	-	-
25	1861	1087	NB	guaiacol	MS, RI, S, O	1.0 (±4%)a	0.7 (±18%)b	0.8 (±11%)b	0.7 (±19%)b	0.0195
26	1872	1285	NB	safrol	MS, RI, O	24.0 (±5%)a	19.9 (±7%)b	15.2 (±5%)c	11.0 (±13%)d	<0.0001
27	1917	n.d. <sup>d</sup>	NB	2-phenylethanol	MS, S, O	10.6 (±10%)	tr <sup>i</sup>	tr <sup>i</sup>	tr <sup>i</sup>	-
28	2010 <sup>c</sup>	1064 <sup>c</sup>	NB	Furaneol <sup>TM</sup>	RI, S, O	-	-	-	-	-
29	2015	1404	NB	methyleugenol	MS, RI, S, O	8.7 (±11%)a	7.2 (±6%)b	6.9 (±10%)b	5.2 (±16%)c	0.0003
30	2041	1273	NB	(E)-cinnamaldehyde	MS, RI, S, O	716 (±9%)a	495 (±6%)b	494 (±5%)b	459 (±10%)b	<0.0001
31	2088	1088	NB	p-cresol	MS, RI, S, O	tr <sup>i</sup>	tr <sup>i</sup>	tr <sup>i</sup>	tr <sup>i</sup>	-
32	2151	1443	NB	cinnamylacetate	MS, RI, O	52.8 (±14%)a	31.6 (±6%)b	7.7 (±15%)c	-	<0.0001
33	2167	1356	NB	eugenol	MS, RI, S, O	13.6 (±16%)b	17.2 (±7%)a	8.9 (±13%)c	4.6 (±17%)d	<0.0001
34	2168 <sup>c</sup>	1128 <sup>c</sup>	A	sotolon	RI, S, O	-	-	-	-	-
35	2244 <sup>c</sup>	1420 <sup>c</sup>	NB	(Z)-isoeugenol	RI, S, O	-	-	-	-	-
36	2286	1337	NB	cinnamyl alcohol	MS, RI, S, O	10.6 (±12%)c	16.7 (±5%)b	30.8 (±10%)a	29.4 (±12%)a	<0.0001
37	2350	1449	NB	(E)-isoeugenol	MS, RI, S, O	1.9 (±18%)a	0.6 (±7%)b	0.8 (±28%)b	-	0.0003
38	2447	1429	NB	coumarin	MS, RI, S, O	30.5 (±12%)	28.9 (±8%)	29.1 (±7%)	27.1 (±12%)	0.4586
39	2525 <sup>c</sup>	1263 <sup>c</sup>	A	phenylacetic acid	RI, S, O	-	-	-	-	-
40	2542	1601	NB	methoxyeugenol <sup>f</sup>	MS, RI, O	5.5 (±19%)b	7.0 (±10%)a	5.7 (±8%)b	3.3 (±25%)c	0.0002
41	2567	1395	A	vanillin	MS, RI, S, O	95.7 (±18%)a	67.8 (±18%)b	63.1 (±5%)b	65.9 (±10%)b	0.0041

<sup>a</sup>Number correspond to those in **Tables 5.2** and **5.4**. <sup>b</sup>Retention indices determined by GC-MS on two different stationary phases (Stabilwax<sup>®</sup> and SAC<sup>TM</sup>-5). <sup>c</sup>Retention indices determined by GCO of CLLE extracts on two different polarity columns (RTX<sup>®</sup>-Wax and RTX<sup>®</sup>-5 SILMS). <sup>d</sup>Not detected. <sup>e</sup>Fraction: acidic (A); neutral-basic (NB). <sup>f</sup>Quantification on SAC<sup>TM</sup>-5 column instead of Stabilwax<sup>®</sup> column. <sup>g</sup>Identification criteria: mass spectra (MS); retention index (RI); reference standard compound (S); odor quality (O) from CLLE extracts. <sup>h</sup>Means (with coefficient of variation in parentheses) followed by different letters are significantly different (*p*-value < 0.05) (n = 4). <sup>i</sup>Trace.

**Table 5.4** Stability of some potent odorants in typical cola based on relative concentration during storage for three months.

no. <sup>a</sup>	compound	stability <sup>b</sup>
2	<i>d</i> -limonene	not stable (temperature dependent)
5	octanal	not stable (temperature dependent)
6	nonanal	not stable (temperature dependent)
10	decanal	not stable (temperature dependent)
11	linalool	not stable (temperature dependent)
25	guaiacol	not stable
30	( <i>E</i> )-cinnamaldehyde	not stable
33	eugenol	not stable (temperature dependent)
38	coumarin	stable
41	vanillin	not stable

<sup>a</sup>Number correspond to those in **Tables 5.2** and **5.3**. <sup>b</sup>Based on relative concentration data.

**Table 5.5** Additional volatile compounds identified in colas stored at various temperatures for three months.

no.	RI <sup>a</sup>			compound	identification <sup>e</sup>	relative concentration (ng/g; ppb) <sup>f</sup>				p-value
	wax	SAC-5	F <sup>c</sup>			initial	5°C	room temp.	40°C	
42	1016	929	NB	$\alpha$ -pinene	MS, RI, S	26.2 ( $\pm$ 7%)a	1.9 ( $\pm$ 8%)b	-	-	<0.0001
43	1039	n.d. <sup>b</sup>	NB	( <i>E</i> )-2-butenal	MS	-	-	8.9 ( $\pm$ 2%)	8.4 ( $\pm$ 11%)	0.2874
44	1048	940	NB	$\alpha$ -fenchene	MS, RI	6.0 ( $\pm$ 5%)a	3.6 ( $\pm$ 2%)c	4.7 ( $\pm$ 5%)b	-	<0.0001
45	1055	942	NB	camphene	MS, RI, S	13.0 ( $\pm$ 6%)a	6.3 ( $\pm$ 9%)b	1.9 ( $\pm$ 10%)c	1.5 ( $\pm$ 10%)c	<0.0001
46	1102	970	NB	$\beta$ -pinene	MS, RI, S	3.6 ( $\pm$ 17%)	-	-	-	-
47	1106	968	NB	2-ethenyltetrahydro-2,6,6-trimethyl-2 <i>H</i> -pyran	MS, RI	7.6 ( $\pm$ 8%)c	6.9 ( $\pm$ 14%)c	9.3 ( $\pm$ 2%)b	16.5 ( $\pm$ 3%)a	<0.0001
48	1138	984	NB	2-carene	MS, RI, S	-	-	-	3.0 ( $\pm$ 11%)	-
49	1142	1005	NB	$\delta$ -3-carene	MS, RI, S	9.1 ( $\pm$ 8%)a	6.6 ( $\pm$ 17%)b	2.2 ( $\pm$ 6%)c	-	<0.0001
50	1159	999	NB	$\alpha$ -phellandrene	MS, RI	14.5 ( $\pm$ 2%)a	4.7 ( $\pm$ 3%)b	0.9 ( $\pm$ 13%)c	0.6 ( $\pm$ 20%)c	<0.0001
51	1166	991	NB	$\beta$ -myrcene	MS, RI, S	190 ( $\pm$ 4%)a	118 ( $\pm$ 4%)b	22.5 ( $\pm$ 14%)c	-	<0.0001
52	1172	1012	NB	1,4-cineol	MS, RI, S	53.0 ( $\pm$ 22%)c	35.3 ( $\pm$ 14%)d	92.3 ( $\pm$ 2%)b	223 ( $\pm$ 2%)a	<0.0001
53	1184	986	NB	2,3-dehydro-1,8-cineol	MS, RI, S	5.0 ( $\pm$ 44%)a	4.2 ( $\pm$ 14%)ab	2.3 ( $\pm$ 19%)b	-	0.0446
54	1206	1002	NB	$\beta$ -phellandrene	MS, RI	17.4 ( $\pm$ 12%)a	5.0 ( $\pm$ 21%)b	-	-	<0.0001
55	1240	1039	NB	( <i>E</i> )- $\beta$ -ocimene	MS, RI	6.5 ( $\pm$ 3%)a	4.3 ( $\pm$ 14%)b	-	-	0.0005
56	1243	1059	NB	$\gamma$ -terpinene	MS, RI, S	1,200 ( $\pm$ 4%)a	767 ( $\pm$ 5%)b	403 ( $\pm$ 2%)c	173 ( $\pm$ 3%)d	<0.0001
57	1253	1049	NB	( <i>Z</i> )- $\beta$ -ocimene	MS, RI	15.8 ( $\pm$ 2%)a	8.9 ( $\pm$ 5%)b	1.9 ( $\pm$ 4%)c	-	<0.0001
58	1279	1086	NB	$\alpha$ -terpinolene	MS, RI, S	381 ( $\pm$ 2%)a	249 ( $\pm$ 5%)b	84.7 ( $\pm$ 2%)c	13.0 ( $\pm$ 3%)d	<0.0001
59	1283	1080	NB	isoterpinolene	MS, RI	4.0 ( $\pm$ 10%)b	2.9 ( $\pm$ 3%)c	6.2 ( $\pm$ 2%)a	-	<0.0001
60	1338	985	NB	6-methyl-5-hepten-2-one	MS, RI, S	2.3 ( $\pm$ 3%)a	1.9 ( $\pm$ 9%)b	1.0 ( $\pm$ 6%)c	-	<0.0001
61	1398	n.d. <sup>b</sup>	NB	2-butoxyethanol	MS, S	1.7 ( $\pm$ 26%)b	1.2 ( $\pm$ 8%)c	1.6 ( $\pm$ 4%)bc	2.1 ( $\pm$ 6%)a	0.0014
62	1409	1100	NB	ion 73(100), 81(56), 43(53), 99(45), 116(29)	MS	-	-	-	1.9 ( $\pm$ 7%)	-

**Table 5.5** (continued)

no.	RI <sup>a</sup>			compound	identification <sup>e</sup>	relative concentration (ng/g; ppb) <sup>f</sup>				p-value
	wax	SAC-5	F <sup>c</sup>			initial	5°C	room temp.	40°C	
63	1432	865	NB	5-methyl-2(3 <i>H</i> )-furanone	MS, RI, S	2.2 (±5%)b	2.0 (±10%)b	2.1 (±8%)b	4.7 (±4%)a	<0.0001
64	1467	829	NB	furfural	MS, RI, S	102 (±4%)c	101 (±16%)c	151 (±1%)b	270 (±8%)a	<0.0001
65	1484	1372	NB	α-copaene or α-cubebene	MS, RI	21.3 (±17%)a	7.1 (±16%)b	3.6 (±10%)b	-	<0.0001
66	1491	n.d. <sup>b</sup>	NB	2-ethyl-1-hexanol	MS	14.8 (±22%)a	4.2 (±12%)b	3.8 (±5%)b	4.3 (±6%)b	<0.0001
67	1505	908	NB	2-acetylfuran	MS, RI, S	20.3 (±6%)c	16.4 (±5%)d	28.4 (±1%)b	33.2 (±3%)a	<0.0001
68	1506	1136	NB	camphor	MS, RI, S	12.4 (±11%)a	9.5 (±3%)b	tr <sup>g</sup>	-	0.0058
69	1521	954	NB	benzaldehyde	MS, RI, S	9.2 (±6%)ab	8.2 (±5%)d	8.8 (±2%)bc	9.5 (±5%)a	0.0077
70	1542	1094	NB	ion 43(100), 81(70), 93(37), 59(35), 96(34)	MS	-	-	-	3.3 (±16%)	-
71	1560	1118	NB	( <i>E</i> )-1-methyl-4-(1-methyl ethyl)-2-cyclohexen-1-ol	MS, RI	30.3 (±3%)a	18.2 (±4%)b	5.1 (±2%)c	3.2 (±18%)d	<0.0001
72	1565	1433	NB	α-bergamotene	MS, RI	9.7 (±13%)a	5.2 (±15%)b	1.6 (±16%)c	-	<0.0001
73	1577	960	NB	5-methyl-furfural <sup>d</sup>	MS, RI	66.7 (±9%)b	67.1 (±6%)b	67.9 (±3%)b	78.0 (±4%)a	0.0042
74	1582	1111	NB	fenchol	MS, RI	367 (±7%)a	232 (±7%)b	189 (±4%)c	161 (±15%)c	<0.0001
75	1610	1064	NB	ethyl levulinate	MS, RI	243 (±4%)a	176 (±8%)b	122 (±2%)c	16.7 (±13%)d	<0.0001
76	1613	1122	NB	myrcenol	MS, RI	18.4 (±5%)b	20.6 (±3%)b	72.2 (±4%)a	18.9 (±15%)b	<0.0001
77	1630	1144	NB	( <i>E</i> )-β-terpineol	MS, RI	289 (±5%)a	300 (±5%)a	185 (±3%)b	28.5 (±15%)c	<0.0001
78	1645	n.d. <sup>b</sup>	NB	β-santalene	MS	7.2 (±8%)a	4.9 (±13%)b	1.9 (±2%)c	-	<0.0001
79	1647	1060	NB	acetophenone	MS, RI, S	-	1.7 (±3%)	1.2 (±6%)	1.5 (±28%)	0.0554
80	1656	1153	NB	ocimenol	MS, RI	3.0 (±3%)c	2.5 (±6%)c	7.5 (±4%)b	28.7 (±14%)a	<0.0001
81	1664	826	A	2-furanmethanol	MS, RI, S	1.7 (±13%)c	1.6 (±20%)c	4.7 (±9%)a	3.5 (±24%)b	<0.0001
82	1661	1448	NB	α-caryophyllene	MS, RI	7.2 (±9%)a	4.2 (±22%)b	-	-	0.0024
83	1670	1434	NB	β-farnesene	MS, RI, S	18.5 (±11%)	17.0 (±17%)	-	-	0.4272

**Table 5.5** (continued)

no.	RI <sup>a</sup>			compound	identification <sup>e</sup>	relative concentration (ng/g; ppb) <sup>f</sup>				p-value
	wax	SAC-5	F <sup>c</sup>			initial	5°C	room temp.	40°C	
<b>84</b>	1677	934	A	5-methyl-2(5 <i>H</i> )-furanone	MS, RI	17.1 (±28%)a	7.7 (±12%)b	9.3 (±6%)b	9.2 (±15%)b	0.0008
<b>85</b>	1680	n.d. <sup>b</sup>	NB	( <i>Z</i> )-β-terpineol	MS	64.7 (±5%)b	87.6 (±5%)a	91.7 (±4%)a	23.2 (±16%)c	<0.0001
<b>86</b>	1717	1496	NB	valencene	MS, RI, S	9.9 (±7%)a	7.8 (±14%)b	-	-	0.0157
<b>87</b>	1723	1507	NB	β-bisabolene	MS, RI, S	192 (±14%)a	113 (±17%)b	28.0 (±18%)c	5.9 (±32%)c	<0.0001
<b>88</b>	1723	n.d. <sup>b</sup>	NB	<i>p</i> -mentha-1,5-dien-8-ol	MS	-	-	8.0 (±9%)a	1.3 (±47%)b	<0.0001
<b>89</b>	1729	1240	NB	carvone	MS, RI	-	6.1 (±8%)a	4.9 (±2%)b	2.7 (±31%)c	<0.0001
<b>90</b>	1748	1468	NB	α-farnesene	MS, RI, S	23.8 (±15%)a	13.5 (±21%)b	3.0 (±19%)c	0.6 (±28%)c	<0.0001
<b>91</b>	1752	1524	NB	δ-cadinene	MS, RI	10.7 (±10%)a	10.2 (±15%)a	3.2 (±14%)b	-	<0.0001
<b>92</b>	1757	1383	NB	geranyl acetate	MS, RI, S	61.8 (±12%)a	33.0 (±8%)b	3.8 (±3%)c	-	<0.0001
<b>93</b>	1762	n.d. <sup>b</sup>	NB	α-gurjunene	MS	9.1 (±14%)a	4.6 (±21%)b	1.4 (±14%)c	-	<0.0001
<b>94</b>	1770	n.d. <sup>b</sup>	NB	citronellol	MS, S	4.1 (±11%)	-	-	-	-
<b>95</b>	1771	1501	NB	( <i>Z</i> )-α-bisabolene	MS, RI	10.5 (±14%)a	10.0 (±15%)a	2.2 (±15%)b	-	<0.0001
<b>96</b>	1771	1178	NB	<i>p</i> -methylacetophenone	MS, RI, S	-	-	1.9 (±7%)b	2.9 (±22%)a	0.0324
<b>97</b>	1777	1157	NB	benzenepropanal	MS, RI, S	6.2 (±4%)	5.9 (±6%)	6.8 (±3%)	6.7 (±17%)	0.1960
<b>98</b>	1780	n.d. <sup>b</sup>	NB	<i>p</i> -mentha-1(7),2-dien-8-ol	MS	11.4 (±4%)a	9.5 (±4%)b	6.9 (±3%)c	-	<0.0001
<b>99</b>	1789	n.d. <sup>b</sup>	NB	geranyl propionate	MS	4.3 (±19%)a	2.6 (±14%)b	-	-	0.0085
<b>100</b>	1849	1199	NB	<i>p</i> -cymen-8-ol	MS, RI	15.5 (±3%)c	24.3 (±5%)b	59.0 (±6%)a	54.9 (±14%)a	<0.0001
<b>101</b>	1867	999	A	hexanoic acid	MS, RI, S	tr <sup>g</sup>	tr <sup>g</sup>	tr <sup>g</sup>	tr <sup>g</sup>	-
<b>102</b>	1933	1230	NB	ion 109(100), 125(89), 97(88), 53(47), 81(43)	MS	199 (±5%)a	154 (±7%)b	157 (±5%)b	146 (±12%)b	0.0002
<b>103</b>	1960	1240	NB	2-methoxybenzaldehyde	MS, RI	11.2 (±9%)	10.7 (±6%)	9.5 (±10%)	9.4 (±19%)	0.1110
<b>104</b>	1979	n.d. <sup>b</sup>	NB	2,6-dimethyl-7-octene-2,6-diol	MS	10.0 (±7%)d	17.5 (±13%)c	63.0 (±8%)a	28.5 (±14%)b	<0.0001
<b>105</b>	1990	1094	A	heptanoic acid	MS, RI, S	tr <sup>g</sup>	-	-	-	-

**Table 5.5** (continued)

no.	RI <sup>a</sup>			compound	identification <sup>e</sup>	relative concentration (ng/g; ppb) <sup>f</sup>				p-value
	wax	SAC-5	F <sup>c</sup>			initial	5°C	room temp.	40°C	
106	2010	n.d. <sup>b</sup>	NB	phenol	MS, S	1.1 (±24%)	0.9 (±10%)	1.1 (±23%)	1.2 (±19%)	0.3354
107	2045	1300	NB	ion 111(100), 43(37), 55(24), 100(16), 93(16)	MS	36.5 (±12%) <sup>c</sup>	33.1 (±9%) <sup>c</sup>	157 (±8%) <sup>b</sup>	359 (±9%) <sup>a</sup>	<0.0001
108	2080	1186	A	octanoic acid	MS, RI, S	tr <sup>g</sup>	tr <sup>g</sup>	tr <sup>g</sup>	tr <sup>g</sup>	-
109	2097	1311	NB	( <i>E</i> )-1,8-terpin / terpin hydrate	MS	56.8 (±5%) <sup>b</sup>	108 (±21%) <sup>b</sup>	1,000 (±8%) <sup>a</sup>	1,110 (±11%) <sup>a</sup>	<0.0001
110	2120	n.d. <sup>b</sup>	NB	( <i>E</i> )- <i>p</i> -menth-2-ene-1,8-diol	MS	3.0 (±20%) <sup>a</sup>	2.0 (±20%) <sup>b</sup>	2.6 (±19%) <sup>ab</sup>	-	0.0496
111	2163	1629	NB	γ-eudesmol	MS, RI	3.3 (±14%)	3.6 (±6%)	3.6 (±19%)	2.8 (±19%)	0.1725
112	2173	1331	NB	( <i>Z</i> )-1,8-terpin	MS	3.2 (±11%) <sup>c</sup>	5.5 (±30%) <sup>c</sup>	70.2 (±11%) <sup>b</sup>	127 (±13%) <sup>a</sup>	<0.0001
113	2193	n.d. <sup>b</sup>	NB	( <i>Z</i> )- <i>p</i> -menth-2-ene-1,8-diol	MS	3.3 (±19%)	3.0 (±31%)	3.9 (±24%)	-	0.2811
114	2199	n.d. <sup>b</sup>	NB	5-acetoxymethyl-2-furaldehyde	MS	1.9 (±18%) <sup>b</sup>	5.7 (±22%) <sup>a</sup>	3.4 (±33%) <sup>b</sup>	2.4 (±35%) <sup>b</sup>	0.0006
115	2216	1683	NB	α-bisabolol	MS, RI	13.4 (±21%) <sup>a</sup>	11.1 (±9%) <sup>a</sup>	6.6 (±20%) <sup>b</sup>	-	0.0021
116	2229	1555	NB	elemicin	MS, RI	13.4 (±23%)	12.9 (±6%)	16.0 (±18%)	12.4 (±19%)	0.2197
117	2267	1523	NB	myristicin <sup>d</sup>	MS, RI	157 (±13%) <sup>a</sup>	116 (±9%) <sup>b</sup>	82.7 (±7%) <sup>c</sup>	52.2 (±14%) <sup>d</sup>	<0.0001
118	2433	2185	NB	<i>n</i> -butylhexadecanoate	MS	20.2 (±34%) <sup>a</sup>	17.2 (±40%) <sup>ab</sup>	10.6 (±48%) <sup>b</sup>	1.9 (±16%) <sup>c</sup>	0.0023
119	2439	1530	NB	<i>o</i> -methoxycinnamaldehyde	MS	127 (±17%) <sup>a</sup>	103 (±8%) <sup>b</sup>	103 (±9%) <sup>b</sup>	84.9 (±10%) <sup>b</sup>	0.0062
120	2474	1619	NB	benzophenone	MS, RI	3.6 (±19%) <sup>a</sup>	1.6 (±40%) <sup>bc</sup>	1.4 (±5%) <sup>c</sup>	2.2 (±4%) <sup>b</sup>	0.0002
121	2505	1250	NB	5-hydroxymethylfurfural	MS, RI	19.6 (±17%) <sup>c</sup>	25.3 (±29%) <sup>c</sup>	41.5 (±9%) <sup>b</sup>	89.8 (±17%) <sup>a</sup>	<0.0001

<sup>a</sup>Retention indices determined on two different stationary phases (Stabilwax<sup>®</sup> and SAC<sup>TM</sup>-5). <sup>b</sup>Not detected. <sup>c</sup>Fraction: acidic (A); neutral-basic (NB). <sup>d</sup>Quantification on SAC<sup>TM</sup>-5 column instead of Stabilwax<sup>®</sup> column. <sup>e</sup>Identification criteria: mass spectra (MS); retention index (RI); reference standard compound (S). <sup>f</sup>Means (with coefficient of variation in parentheses) followed by different letters are significantly different (*p*-value < 0.05) (*n* = 4). <sup>g</sup>Trace.

## VII. REFERENCES

- Baxter, R.L.; Laurie, W.A.; McHale, D. Transformations of monoterpenoids in aqueous acids: The reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid. *Tetrahedron* **1978**, *34*, 2195-2199.
- Buttery, R.G.; Ling, L.C.; Light, D.M. Tomato leaf volatile aroma components. *J. Agric. Food Chem.* **1987**, *35*, 1039-1042.
- Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006-1009.
- Clark, B.C., Jr.; Chamblee, T.S. Acid-catalyzed reactions of citrus oils and other terpene-containing flavors. In *Off-Flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier: Amsterdam, 1992; pp 229-285.
- Clark, B.C., Jr.; Powell, C.C.; Radford, T. The acid catalyzed cyclization of citral. *Tetrahedron* **1977**, *33*, 2187-2191.
- Cori, O.; Chayet, L.; Perez, L.M.; Bunton, C.A.; Hachey, D. Rearrangement of linalool, geraniol, and nerol and their derivatives. *J. Org. Chem.* **1986**, *51*, 1310-1316.
- Dodge, F.D. Some derivatives of coumarin. *J. Am. Chem. Soc.* **1916**, *38*, 446-457.
- Elmore, J.S.; Erbahadir, M.A.; Mottram, D.S. Comparison of dynamic headspace concentration on Tenax with solid phase microextraction for the analysis of aroma volatiles. *J. Agric. Food Chem.* **1997**, *45*, 2638-2641.
- Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237-241.
- Grein, B.; Schmidt, G.; Full, G.; Winterhalter, P.; Schreier, P. 2-Formylmethyl-2-methyl-5-(1-hydroxy-1-methylethyl)-tetrahydrofuran: Major volatile product of the water mediated oxidative decomposition of citral. *Flavour Frag. J.* **1994**, *9*, 93-98.
- Hida, Y.; Matsumoto, M.; Kudo, K.; Imamura, T.; Ikeda, N. Identification of beverages and beverage stains by GC/MS using aroma components as indicators. *Int. J. Legal. Med.* **1998**, *111*, 13-16.
- IBISWorld. Global Soft Drink & Bottled Water Manufacturing. *Global Industry Report C1124-GL* [Online], November 9, 2011, p 14. IBISWorld.  
<http://clients.ibisworld.com/globalindustry/default.aspx?indid=420> (accessed January 12, 2012).

- Jirovetz, L.; Buchbauer, G.; Stoilova, I.; Stoyanova, A.; Krastanov, A.; Schmidt, E. Chemical composition and antioxidant properties of clove leaf essential oil. *J. Agric. Food Chem.* **2006**, *54*, 6303-6307.
- Kaczanowska, A. Carbonated soft drink production in the US. *IBISWorld Industry Report 31211a* [Online], December, 2010b, p 12. IBISWorld.  
<http://www.ibisworld.com/industryus/default.aspx?indid=285> (accessed December 9, 2010).
- Kaczanowska, A. Soft drink production in the US. *IBISWorld Industry Report 31211* [Online], August, 2010a, pp 13-14. IBISWorld.  
<http://www.ibisworld.com/industryus/default.aspx?indid=284> (accessed December 9, 2010).
- Kimura, K.; Iwata, I.; Nishimura, H. Relationship between acid-catalyzed cyclization of citral and deterioration of lemon flavor. *Agric. Biol. Chem.* **1982**, *46*, 1387-1389.
- Kimura, K.; Nishimura, H.; Iwata, I.; Mizutani, J. Deterioration mechanism of lemon flavor. 2. Formation mechanism of off-odor substances arising from citral. *J. Agric. Food Chem.* **1983a**, *31*, 801-804.
- Kimura, K.; Nishimura, H.; Iwata, I.; Mizutani, J. Identification of acidic substances from deteriorated citral and effects of antioxidants on their formation. *Agric. Biol. Chem.* **1983b**, *47*, 1661-1663.
- Lee, H.S.; Nagy, S. Chemical degradative indicators to monitor the quality of processed and stored citrus products. In *Chemical Markers for Processed and Stored Foods*; Lee, T-C., Kim, H-J., Eds.; ACS Symposium Series 631; American Chemical Society: Washington, DC, 1996; pp 86-106.
- Lo, C-Y.; Li, S.; Wang, Y.; Tan, D.; Pan, M-H.; Sang, S.; Ho, C-T. Reactive dicarbonyl compounds and 5-(hydroxymethyl)-2-furfural in carbonate beverages containing high fructose corn syrup. *Food Chem.* **2008**, *107*, 1099-1105.
- Lorjaroenphon, Y.; Hausch, B.J.; Cadwallader, K.R. Formation and stability of 2,3-dehydro-1,8-cineol in a model carbonated beverage system. Proceedings of 13<sup>th</sup> Weurman Flavor Research Symposium, Zaragoza, Spain, September 27-30, 2011; in press.
- Nagy, S.; Randall, V. Use of furfural content as an index of storage temperature abuse in commercially processed orange juice. *J. Agric. Food Chem.* **1973**, *21*, 272-275.
- O'Mahony, M. Understanding discrimination tests: a user-friendly treatment of response bias, rating and ranking R-index tests and their relationship to signal detection. *J. Sensory Stud.* **1992**, *7*, 1-47.

- Ohta, T.; Morimitsu, Y.; Sameshima, Y.; Samuta, T.; Ohba, T. Transformation from geraniol, nerol and their glucosides into linalool and  $\alpha$ -terpineol during Shochu distillation. *J. Ferment. Bioeng.* **1991**, *72*, 347-351.
- Peacock, V.E.; Kuneman, D.W. Inhibition of the formation of  $\alpha$ -*p*-dimethylstyrene and *p*-cymen-8-ol in a carbonated citral-containing beverage system. *J. Agric. Food Chem.* **1985**, *33*, 330-335.
- Pendergrast, M. *For God, Country and Coca-Cola*; Basic Books: New York, 2000; pp 456-460.
- Schieberle, P.; Ehrmeier, H.; Grosch, W. Aroma compounds resulting from the acid catalysed breakdown of citral. *Z. Lebensm. Unters. Forsch.* **1988**, *187*, 35-39.
- Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797-800.
- Schieberle, P.; Grosch, W. Potent odorants resulting from the peroxidation of lemon oil. *Z. Lebensm. Unters. Forsch.* **1989**, *189*, 26-31.
- Shaw, P.E.; Tatum, J.H.; Berry, R.E. Acid-catalyzed degradation of *d*-fructose. *Carbohydr. Res.* **1967**, *5*, 266-273.
- Shillinglaw, C.A.; Levine, M. Control of oxidative flavors in beverages. *J. Food Sci.* **1943**, *8*, 453-463.
- Steen, D.P. Carbon dioxide, carbonation and principles of filling technology. In *Carbonated Soft Drinks: Formulation and Manufacture*; Steen, D.P., Ashurst, P.R., Eds.; Blackwell Publishing: Chennai, India, 2006; pp 112-143.
- Tchudi, S.N. *Soda Poppery*; Charles Scribner's sons: New York, 1986; pp 32-34.
- Ueno, T.; Kiyohara, S.; Ho, C-T.; Masuda, H. Potent inhibitory effects of black tea theaflavins on off-odor formation from citral. *J. Agric. Food Chem.* **2006**, *54*, 3055-3061.
- Ueno, T.; Masuda, H.; Ho, C-T. Combined inhibitory effects of catechins with Fe<sup>3+</sup> on the formation of potent off-odorants from citral. In *Phenolic Compounds in Foods and Natural Health Products*; Shahidi, F., Ho, C-H., Eds.; ACS Symposium Series 909; American Chemical Society: Washington, DC, 2005; pp 129-142.
- Ueno, T.; Masuda, H.; Ho, C-T. Formation mechanism of *p*-methylacetophenone from citral via a tert-alkoxy radical intermediate. *J. Agric. Food Chem.* **2004**, *52*, 5677-5684.
- van den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463-471.

Watcharananun, W.; Cadwallader, K.R.; Huangrak, K.; Kim, H.; Lorjaroenphon, Y.  
Identification of predominant odorants in Thai desserts flavored by smoking with “Tian  
Op”, a traditional Thai scented candle. *J. Agric. Food Chem.* **2009**, *57*, 996-1005.

Wiley, R.C.; Louie, M.K.; Sheu, M-J. Studies on turpentine-like off-odor in cola. *J. Food Sci.*  
**1984**, *49*, 485-488.

## CHAPTER 6

### SUMMARY, CONCLUSIONS, IMPLICATIONS, AND SUGGESTIONS FOR FURTHER RESEARCH

Cola commands the highest market share among the various types of commercial carbonated beverages. Despite its popularity, there is limited information about the flavor chemistry of cola. In fact, the recipe of Coca-Cola is the one of the world's most famous trade secrets. Natural flavors, which may include essential oils and vanilla extract, are listed on the ingredient labels of the major brands of commercial colas. These ingredients are the sources of cola aroma, and this flavor blend results in uniqueness and complexity of the product. While cola flavor is highly desirable, it, unfortunately, is unstable because of the acid-catalyzed degradation of certain volatile components of the essential oils used in the flavorings. These reactions may lead to reduction in product quality.

Thus, the main objective of this study was to characterize the acid-catalyzed reactions which may cause changes in the volatile aroma-active compounds of stored colas. It was hypothesized that both degradation of desirable odorants and formation of storage-induced off-odorants cause flavor changes in stored cola. To investigate the loss of desirable odorants, the potent odorants of typical cola were identified and characterized first. Then, the degradation of potent odorants and formation of storage-induced odorants could be investigated.

The aroma-active compounds in the top three US brands of regular colas were identified by gas chromatography-olfactometry (GCO) and GC-mass spectrometry (GC-MS). Aroma extract dilution analysis (AEDA), which is one type of GCO technique, was also performed to rank the potency of aroma compounds. Eugenol (spicy, clove-like) and coumarin (sweet note)

were the most potent odorants in all typical colas. The additional highly potent odorants in some colas were guaiacol (smoky) and linalool (floral), while 1,8-cineol (eucalyptus-like) was a moderately potent odorant in all colas. However, identification of potent odorants by application of AEDA has limitations since the interaction between odorants and the food matrix is not taken into account.

To correct the lack of interaction information, odor activity value (OAV), which is the ratio of concentration to odor threshold, was calculated for each specific odorant. Stable isotope dilution analysis (SIDA) was applied for accurate quantification which resulted in more accurate OAVs. 1,8-Cineol, (*R*)-(-)-linalool, and octanal (citrus-like) were identified as key potent odorants which contribute to the overall aroma of cola followed by nonanal, (*S*)-(+)-linalool, decanal, and (*R*)-(+)-limonene. However, the actual potency of aromas may not necessarily correlate with the odor threshold concept of OAV. It is also necessary to validate these analytical data in the real food matrix.

Cola aroma reconstitution model was constructed based on the quantification results. Twenty high purity aroma compounds were blended with a mimic cola matrix. The model had cola-like aroma characteristic, and the aroma attributes of the cola model after adjusting the balance of aroma compounds did not differ from authentic cola. In addition, the omission of one group of compounds consisting of methyleugenol, (*E*)-cinnamaldehyde, eugenol, (*Z*)- and (*E*)-isoeugenols resulted in detectable change in overall aroma. However, no differences were detected between the complete model and omission models based on omitting individual components of this group. These results implied that a balance of numerous odorants was responsible for the characteristic aroma of cola-flavored carbonated beverages.

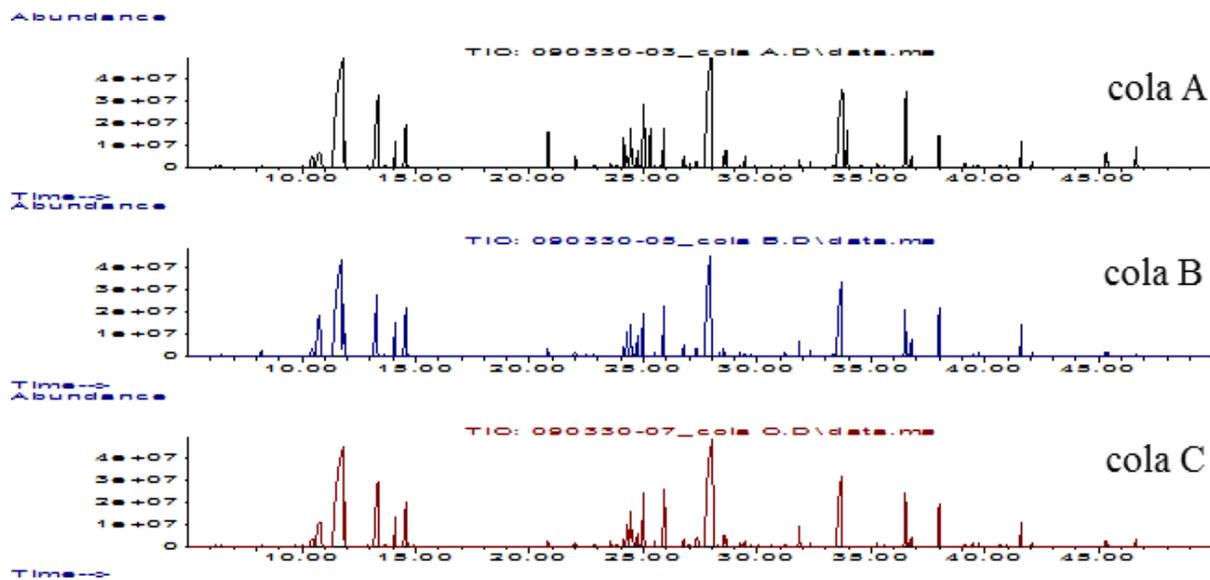
The degradation of potent odorants and formation of storage-induced odorants were investigated in colas stored at 5°C, room temperature, and 40°C for three months. The aroma of cola stored at 40°C (which is an unusually high temperature) was found to be significantly different from typical cola by application of sensory R-index ranking test and descriptive analysis. AEDA results indicated no unique potent odorants were formed in stored colas as compared to the initial cola sample (day 0). In comparison, the potency of linalool and octanal in storage-abused cola decreased, while *p*-cresol (dung-like) dramatically increased. These volatile changes were the results of acid-catalyzed reactions.

The semi-quantification data also indicated significant loss of most of the typical potent odorants by acid-catalyzed reactions and demonstrated that deterioration rates were a function of storage temperature. In addition, limonene, which is the most abundance aroma-active compound in cola, was also converted to odorless volatiles or compounds having only minimal odor response, such as (*Z*)- and (*E*)-1,8-terpines. This loss of limonene may affect the partition of other volatile aromas and cause flavor changes. Coumarin which was the potent odorants of typical cola was, however, stable during storage.

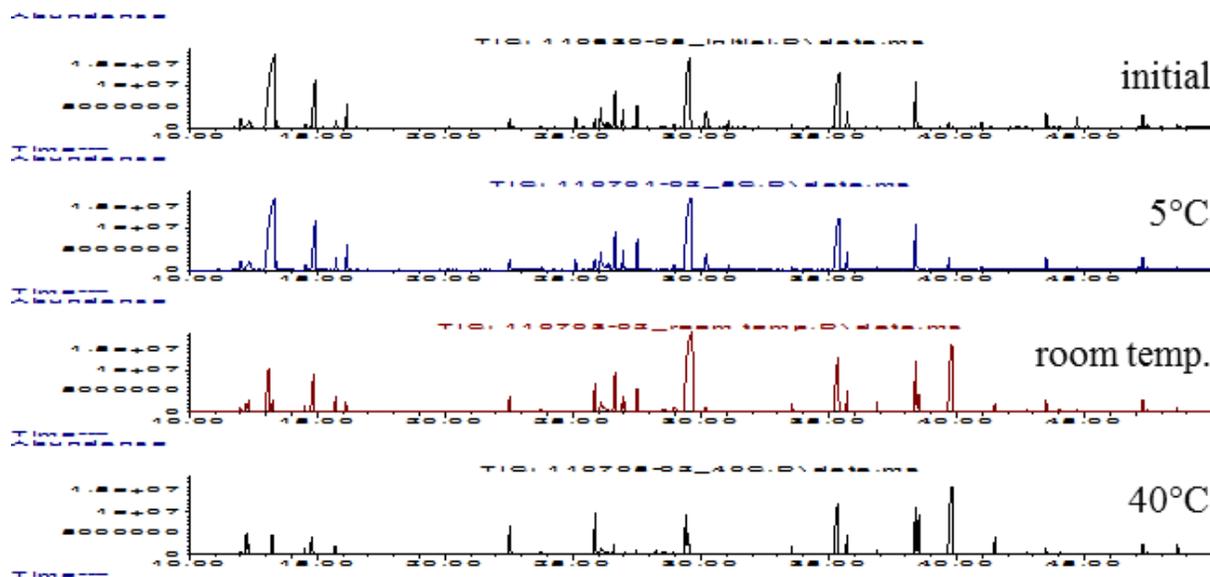
These analytical results indicated that aroma changes of stored cola were due to both degradation of typical potent odorants and formation of storage-induced flavors. Additionally, 2-carene and two unidentified components were the unique volatiles which could be used as chemical markers to indicate storage temperature abuse. The present study expands the current knowledge of acid-catalyzed reactions of aroma compounds in a real food system, which may ultimately lead to improvements in the flavor and shelf-life stability of cola-flavored carbonated beverages.

Formation mechanism of the off-odor compound, *p*-cresol, in cola is still unclear. It is proposed to be an oxidation product of citral degradation, but the oxygen concentration in carbonated beverages is very low. Further study on the formation of *p*-cresol in a carbonated beverage model system is recommended. The hypothesis that this odorant and other oxidation products, such as *p*-methylacetophenone, may be derived by other oxygen-independent reactions should be evaluated. Omission study of storage-induced aromas is also necessary to investigate how changes in specific volatile compounds influence the overall aroma. In addition, the study of the flavor release of cola aroma compounds will answer the question about difference between orthonasal (aroma-by-nose) and retronasal (aroma-by-mouth) perception of cola flavor.

**APPENDIX A**  
**TOTAL ION CHROMATOGRAMS OF**  
**COLA-FLAVORED CARBONATED BEVERAGES**



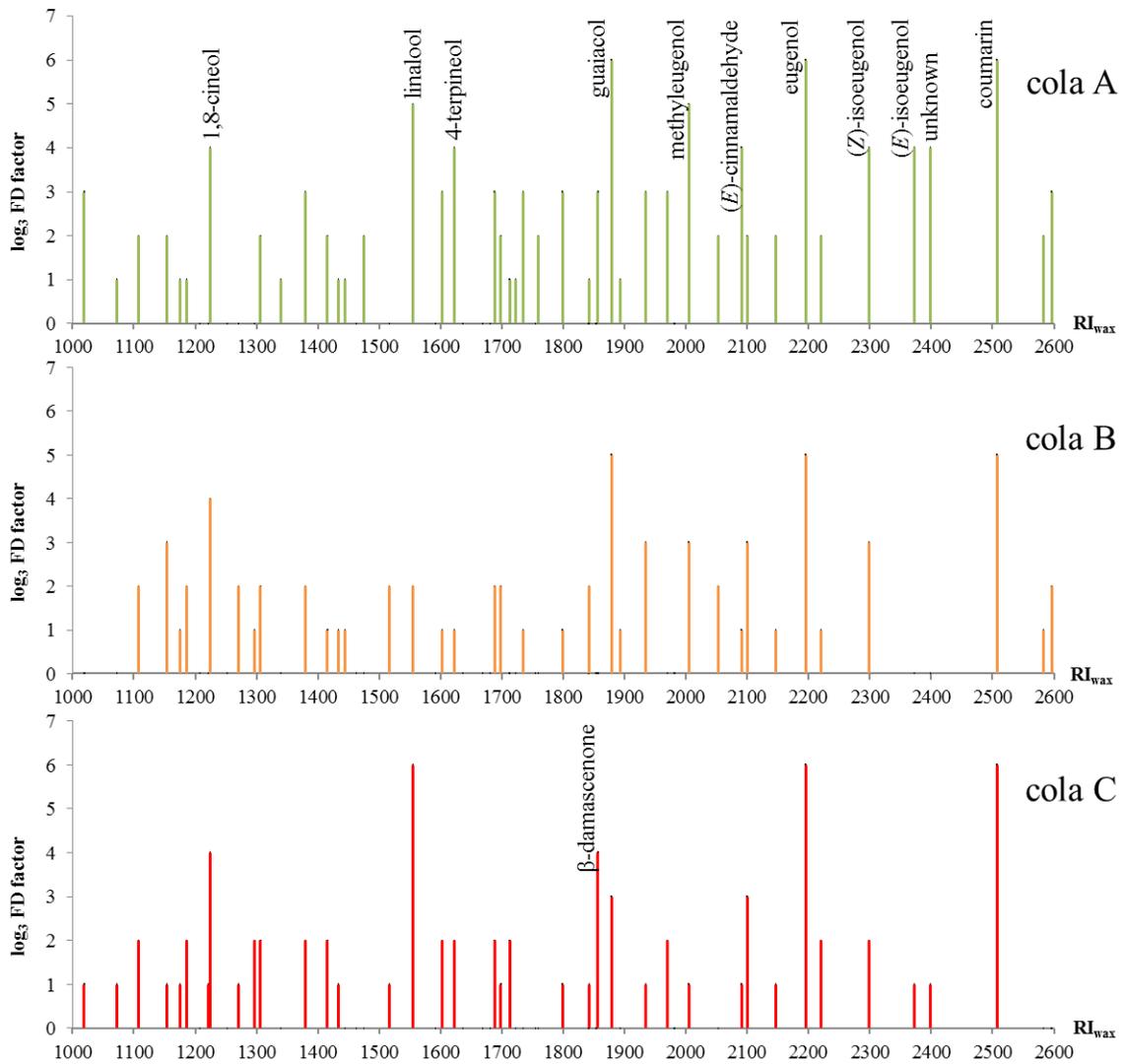
**Figure A.1** Total ion chromatograms of cola-flavored carbonated beverages (**Chapter 3**).



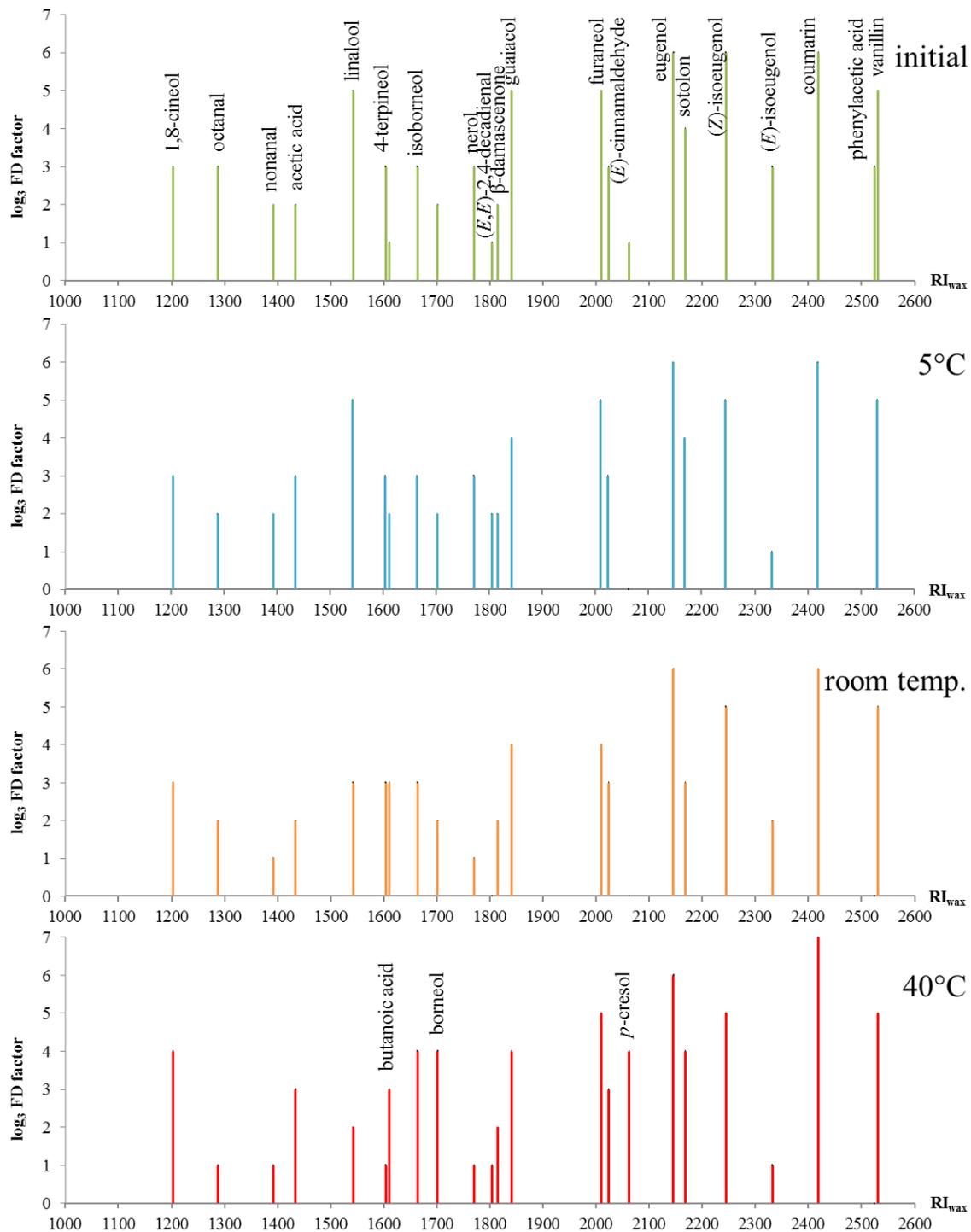
**Figure A.2** Total ion chromatograms of stored colas (**Chapter 5**).

## APPENDIX B

### FLAVOR DILUTION CHROMATOGRAMS OF POTENT ODORANTS IN COLA-FLAVORED CARBONATED BEVERAGES



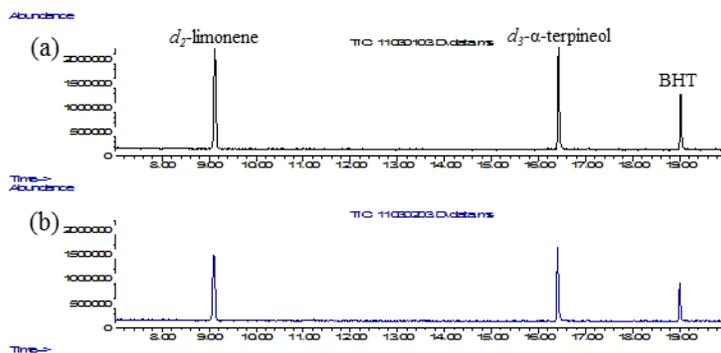
**Figure B.1** Flavor dilution chromatograms of potent odorants in typical cola-flavored carbonated beverages (**Chapter 3**).



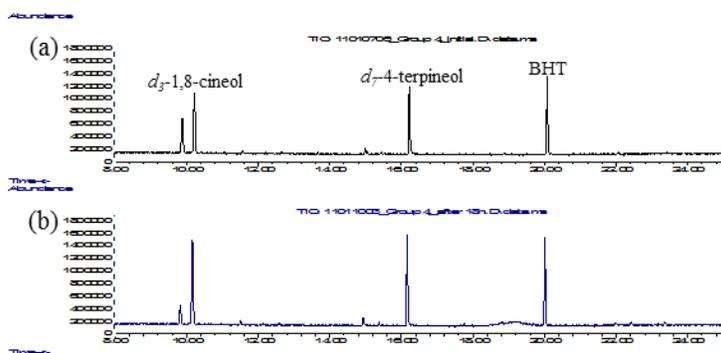
**Figure B.2** Flavor dilution chromatograms of potent odorants ( $FD \geq 9$ ) in colas stored at various temperatures for 3 months (**Chapter 5**).

## APPENDIX C

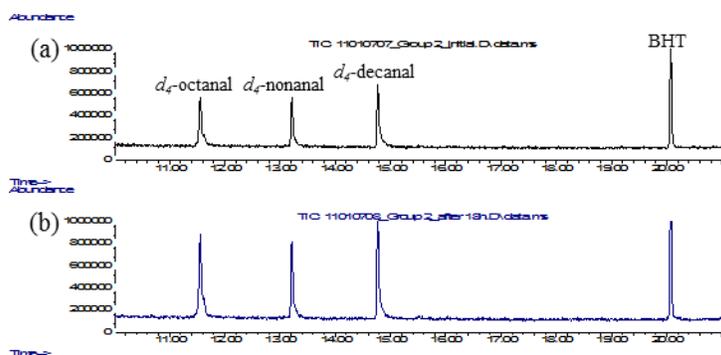
### STABILITY OF ISOTOPICALLY LABELED STANDARDS IN AQUEOUS WEAK ACID SOLUTION



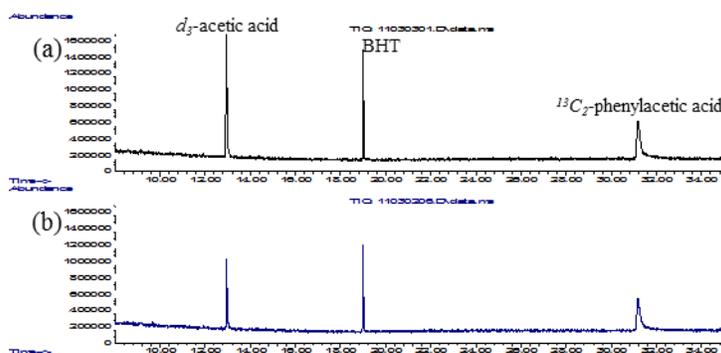
**Figure C.1** Total ion chromatograms of [ $^2H_2$ ]-limonene (**I-8**) and [ $^2H_3$ ]- $\alpha$ -terpineol (**I-31**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



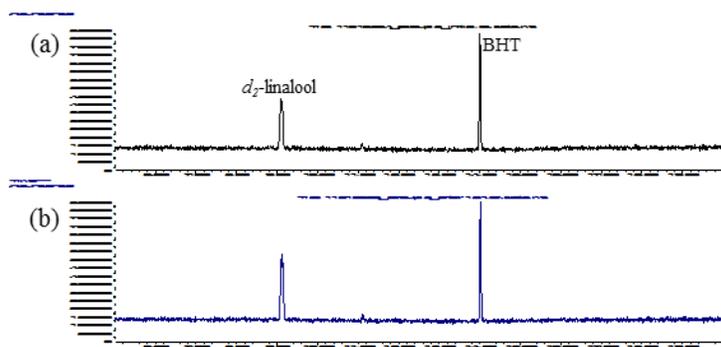
**Figure C.2** Total ion chromatograms of [ $^2H_3$ ]-1,8-cineol (**I-9**) and [ $^2H_7$ ]-4-terpineol (**I-25**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



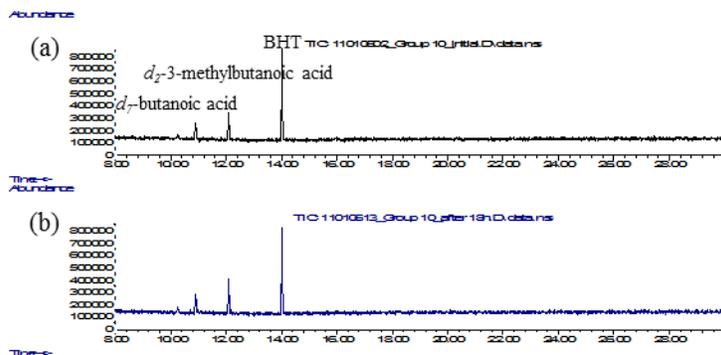
**Figure C.3** Total ion chromatograms of  $[^2H_4]$ -octanal (**I-13**),  $[^2H_4]$ -nonanal (**I-16**), and  $[^2H_4]$ -decanal (**I-21**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



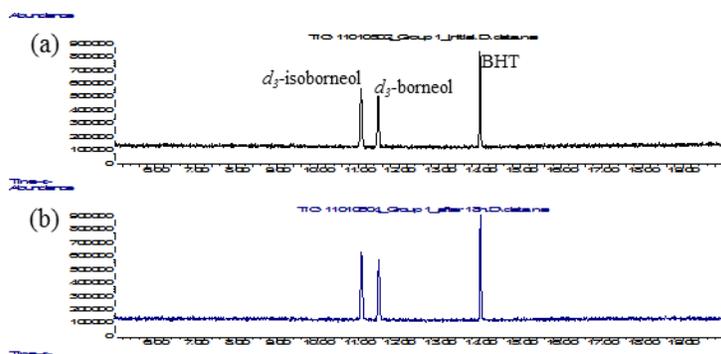
**Figure C.4** Total ion chromatograms of  $[^2H_3]$ -acetic acid (**I-19**) and  $[^{13}C_2]$ -phenylacetic acid (**I-57**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



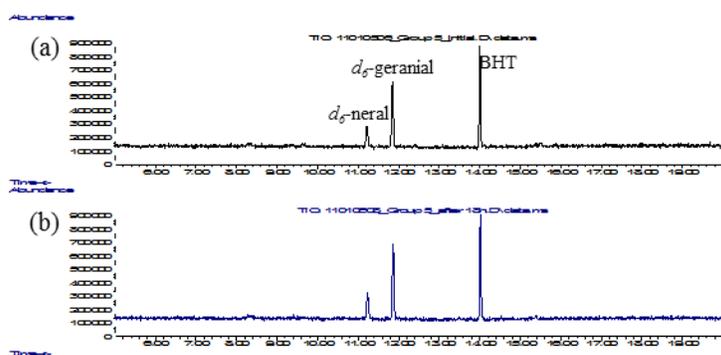
**Figure C.5** Total ion chromatograms of  $[^2H_2]$ -linalool (**I-22**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



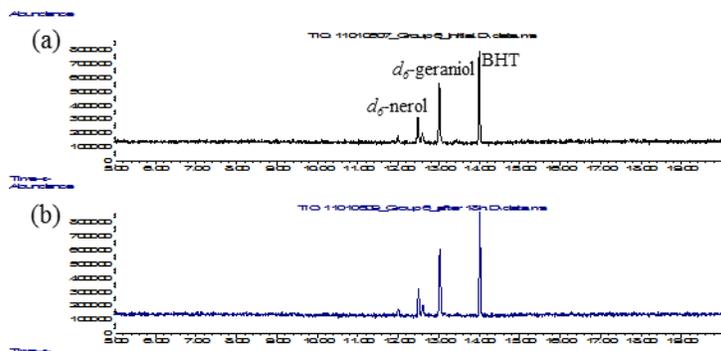
**Figure C.6** Total ion chromatograms of [ $^2H_7$ ]-butanoic acid (**I-26**) and [ $^2H_2$ ]-3-methylbutanoic acid (**I-28**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



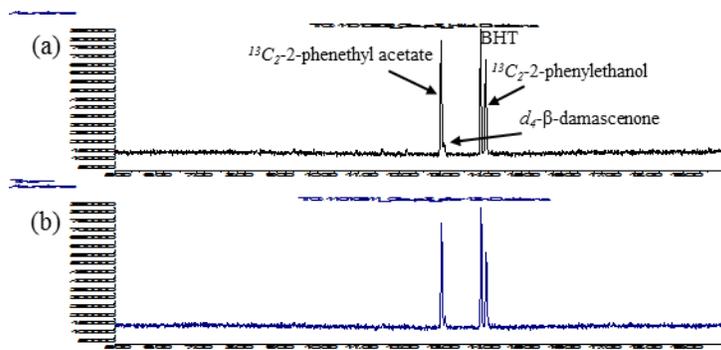
**Figure C.7** Total ion chromatograms of [ $^2H_3$ ]-isoborneol (**I-29**) and [ $^2H_3$ ]-borneol (**I-32**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



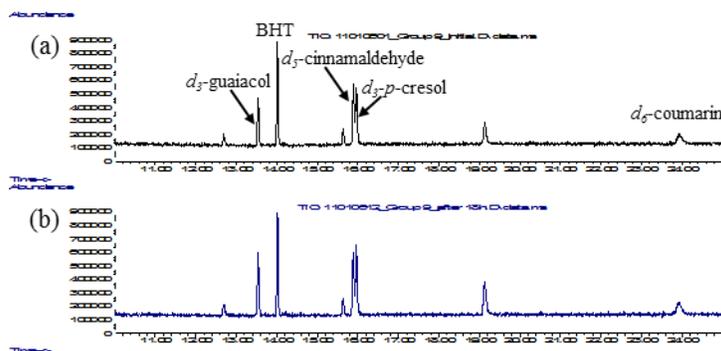
**Figure C.8** Total ion chromatograms of [ $^2H_6$ ]-neral (**I-30**) and [ $^2H_6$ ]-geranial (**I-34**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



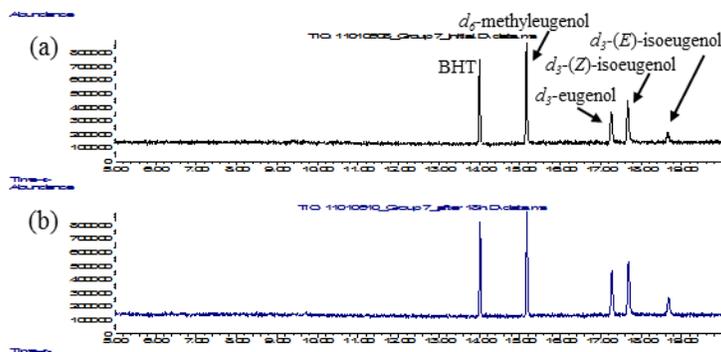
**Figure C.9** Total ion chromatograms of [ $^2H_6$ ]-nerol (**I-36**) and [ $^2H_6$ ]-geraniol (**I-39**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



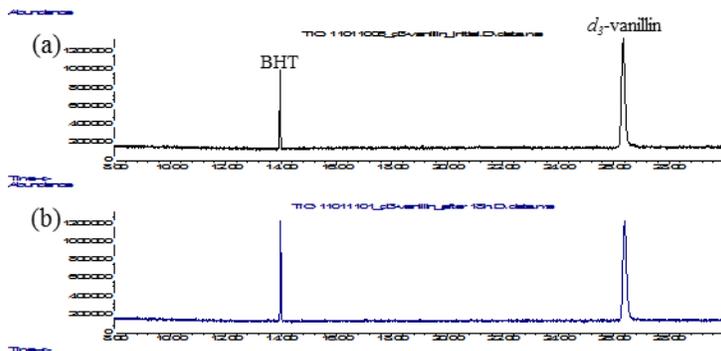
**Figure C.10** Total ion chromatograms of [ $^{13}C_2$ ]-2-phenethyl acetate (**I-37**), [ $^2H_4$ ]- $\beta$ -damascenone (**I-40**), and [ $^{13}C_2$ ]-2-phenylethanol (**I-43**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



**Figure C.11** Total ion chromatograms of [ $^2H_3$ ]-guaiacol (**I-41**), [ $^2H_5$ ]-cinnamaldehyde (**I-48**), [ $^2H_3$ ]-*p*-cresol (**I-49**), and [ $^2H_6$ ]-coumarin (**I-56**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



**Figure C.12** Total ion chromatograms of [ $^2H_6$ ]-methyl Eugenol (**I-46**), [ $^2H_3$ ]-eugenol (**I-51**), [ $^2H_3$ ]-(*Z*)-isoeugenol (**I-53**), and [ $^2H_3$ ]-(*E*)-isoeugenol (**I-54**): before (a) and after (b) extraction for 18 h (**Chapter 4**).



**Figure C.13** Total ion chromatograms of [ $^2H_3$ ]-vanillin (**I-58**): before (a) and after (b) extraction for 18 h (**Chapter 4**).

## APPENDIX D

### MEANS AND STANDARD DEVIATIONS FOR DESCRIPTIVE ANALYSIS RESULTS

**Table D.1** Sample means for typical cola-flavored carbonated beverages (**Chapter 3**).

attribute	intensity <sup>a</sup>			p-value
	cola A	cola B	cola C	
lemon-lime	5.3 ± 1.0a	4.2 ± 1.3b	5.7 ± 1.1a	0.0177
orange	3.5 ± 1.2	2.9 ± 1.1	3.2 ± 0.9	0.4438
brown spice	4.2 ± 1.2	3.3 ± 1.4	3.5 ± 1.1	0.3027
herbal	2.7 ± 0.8	3.5 ± 1.6	2.4 ± 0.6	0.0857
vanilla	2.8 ± 0.9	2.5 ± 0.8	2.7 ± 1.0	0.7719
caramel	3.3 ± 1.0	2.7 ± 1.2	2.7 ± 1.3	0.4590
cooling	4.5 ± 1.2	4.3 ± 1.2	3.8 ± 1.3	0.4726
pine	3.0 ± 1.2	3.5 ± 1.0	3.8 ± 1.3	0.3847

<sup>a</sup>Means and standard deviations followed by different letters are significantly different ( $p$ -value < 0.05) ( $n = 10$ ; 7 females and 3 males; 22-38 years).

**Table D.2** Sample means for cola models compared to authentic cola (**Chapter 4**).

attribute	intensity <sup>a</sup>			p-value
	authentic cola	original model	re-balanced model	
lemon-lime	4.8 ± 0.7	4.4 ± 0.6	4.4 ± 0.5	0.1535
orange	3.6 ± 0.5	3.4 ± 0.6	3.3 ± 0.5	0.5866
brown spice	3.7 ± 0.5	3.7 ± 0.7	3.7 ± 0.6	0.9487
herbal	3.1 ± 1.0b	4.3 ± 1.0a	3.8 ± 0.7ab	0.0178
vanilla	2.9 ± 0.8	2.6 ± 0.7	2.7 ± 0.8	0.5517
caramel	2.6 ± 0.3	2.3 ± 0.4	2.4 ± 0.4	0.3091
cooling	4.0 ± 0.5	3.8 ± 0.6	3.8 ± 0.6	0.5367
pine	3.4 ± 0.6b	4.2 ± 0.6a	3.9 ± 0.5ab	0.0133

<sup>a</sup>Means and standard deviations followed by different letters are significantly different ( $p$ -value < 0.05) (n = 10; 7 females and 3 males; 23-45 years).

**Table D.3** Sample means for omission model compared to complete model (**Chapter 4**).

attribute	intensity <sup>a</sup>		p-value
	complete model	group 5-omission model	
lemon-lime	4.1 ± 0.4	4.2 ± 0.4	0.7989
orange	3.5 ± 0.6	3.4 ± 0.5	0.4367
brown spice	3.6 ± 0.8	3.5 ± 0.8	0.3788
herbal	3.6 ± 0.9	3.6 ± 0.9	0.9242
vanilla	2.4 ± 0.4	2.4 ± 0.3	0.8850
caramel	2.2 ± 0.5	2.1 ± 0.5	0.3282
cooling	3.7 ± 0.8	3.9 ± 0.7	0.2351
pine	4.0 ± 0.7	3.7 ± 0.5	0.2931

<sup>a</sup>Means and standard deviations (n = 8; 5 females and 3 males; 26-45 years).

**Table D.4** Sample means for colas stored at various temperatures for 3 months (**Chapter 5**).

attribute	intensity <sup>a</sup>				<i>p</i> -value
	typical cola	5°C	room temp.	40°C	
lemon-lime	5.3 ± 0.9a	5.5 ± 1.3a	3.7 ± 1.1b	3.7 ± 1.2b	0.0005
orange	3.9 ± 0.9	4.0 ± 1.6	3.7 ± 1.1	3.5 ± 1.2	0.8434
brown spice	3.9 ± 0.8	4.5 ± 1.5	4.0 ± 0.7	3.7 ± 1.2	0.4475
herbal	2.9 ± 0.9	3.2 ± 1.1	3.4 ± 1.3	2.8 ± 0.7	0.5097
vanilla	3.5 ± 1.0	3.3 ± 0.9	2.8 ± 0.4	3.3 ± 1.1	0.3728
caramel	3.0 ± 1.1b	2.6 ± 0.7b	3.0 ± 1.1b	3.9 ± 0.8a	0.0200
cooling	4.6 ± 0.8	4.1 ± 1.2	4.0 ± 0.9	4.2 ± 1.1	0.5001
pine	3.5 ± 1.0	3.3 ± 1.0	3.4 ± 1.1	4.2 ± 0.6	0.1644

<sup>a</sup>Means and standard deviations followed by different letters are significantly different (*p*-value < 0.05) (n = 8; 5 females and 3 males; 22-38 years).