# VOLATILE COMPOUNDS IN VINE TEA (*Ampelopsis grossedentata*)

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## ABSTRACT

Vine tea (*Ampelopsis grossedentata*) is a Chinese herbal tea, rich in the natural antioxidant dihydromyricetin, that has multiple health properties and potential food and beverage applications. However, there is little information available on vine tea aroma, color and sensory characteristics. In this study, volatile components of vine tea infusions were identified by headspace solid-phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). Commercial vine teas samples were brewed with distilled water and Blacksburg (VA, USA) tap water and analyzed in triplicate. Vine tea infusions had acidic pH values and dark, reddish-yellow color. Type of water and vine tea sample both affected the overall volatile chemical composition of vine tea infusions. A total of fifty-six volatile components were identified vine tea infusions. However, only seven aldehydes (hexanal, (E)-2-hexenal, (Z)-4-heptenal, nonanal, (E,Z)-2,4-heptadienal, (E,E)-2,4-heptadienal, and β-cyclocitral), two ketones (6-methyl-5-hepten-2-one and β-ionone), and one alcohol (1-penten-3-ol) were identified in more than 90% of all vine tea infusions. Results of this study may help further investigations in chemical and sensorial characteristics of vine tea, and the development of new healthy products.

**Keywords: vine tea, *Ampelopsis grossedentata*, flavor, GC-MS.**

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## GENERAL AUDIENCE ABSTRACT

Herbal teas have been consumed worldwide because of their health properties and pleasant taste. Vine tea (*Ampelopsis grossedentata*) is a healthy herbal tea traditionally used in Chinese herbal medicine, rich in natural antioxidant dihydromyricetin. Recently, the food industry has observed an increasing number of consumers searching for healthy products for better quality of life. Vine tea has multiple health properties and potential food and beverage applications. The objective of this study was to identify the volatile components present in commercial vine tea samples. Both distilled water and Blacksburg (VA, USA) tap water were used for preparing vine tea infusions, which were characterized by acidic pH values and dark, reddish-yellow color. The overall volatile profile of vine tea infusions was affected by both type of water and vine tea sample. A total of fifty-six volatile components were identified in vine tea infusions, but only ten of them were present in more than 90% of all infusions. Results of this study may help further investigations in chemical and sensorial characteristics of vine tea, and the development of new healthy products.

**Keywords: vine tea, *Ampelopsis grossedentata*, flavor, GC-MS.**

## DEDICATION

This work is dedicated to my parents, Suzete Vieira and Aparecido Carneiro, who always believed a good education is the greatest gift they could give me and my siblings, since knowledge is something no one can ever take away from us.

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# CHAPTER 1

# INTRODUCTION

The interest of commercial, academic and governmental sectors in functional food development has increased over the past few years as a result of health claims acceptability by consumers who believe healthier products increase their quality of life (Jones & Jew, 2007).

Consumption of tea and tea-based products has increased in many parts of the world, especially because consumers have been attracted by their stimulant and health properties (Heck & Gonzalez, 2009). Tea is a universal beverage with long historic and economic importance, and it was first primarily consumed in China due to its medicinal properties (Gascoyne et al., 2014). Nowadays, tea is present in almost 80% of all households in United States and is the second most consumed beverage in the world, behind only water (Heck & Gonzalez, 2009; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Sharangi, 2009; Tea Association of the United States of America, 2015).

White, green, yellow, wulong (or oolong), black and Pu er (or Pu-erh) teas come from different methods of processing leaves of the tea plant (*Camellia sinensis* L.) and they are known as "non-herbal teas" (Gascoyne et al., 2014; Heck & Gonzalez, 2009, Sharangi, 2009). Teas brewed from the leaves, seeds, flowers, fruits, roots or stems of other plant species instead of *Camellia sinensis* L. are named "herbal teas" and can also be denoted "tisanes" (Zhao et al., 2013; Heck & Gonzalez, 2009; Lasekan & Lasekan, 2012).

Vine tea (*Ampelopsis grossedentata*) is a healthy herbal tea, rich in natural antioxidant dihydromyricetin (ampelopsin), and whose dried leaves and stems have been traditionally used in Chinese herbal medicine (Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009; Ye, Wang, Duncan, Eigel, & O'Keefe, 2015; X. J. Zheng, Xiao, Zeng, Sun, Lei, Dong, et al., 2014)

Although vine tea has been considered a health tea, its consumption is still not very popular in Western countries such as United States, where it is not well known. However, the number of researchers studying vine tea health properties, chemical components and possible applications has been increasing significantly in the past few years.

Tschiggerl and Bucar (2012) reported there is a dearth of qualitative and quantitative information of volatile components in herbal tea infusions and the changes of their volatile composition due to tea preparation process. Accordingly, there is still a lack of information about flavor volatile components of vine tea in herbal tea literature. Acceptability and consumer quality of herbal tea will depend on the aroma and flavor quality of herbal teas, so it is important to characterize aroma compounds in new teas so evaluation of aroma quality can begin.

Thus, the objective of this research was to identify the volatile components present in vine tea (*Ampelopsis grossedentata*) infusions, brewed with distilled water and Blacksburg (VA, USA) tap water, using headspace solid-phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). Results of this study may help further investigations in chemical and sensorial characteristics of vine tea, and the development of new, healthy products.

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# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Teas and herbal teas

Tea is the most consumed beverage worldwide after water (CBI, 2015; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Sharangi, 2009; Zihan, Xueli, Dong, Huan, Xiaosong, & Jihong, 2013). The main categories of tea consumed worldwide are black, green and oolong tea, with black tea being the most consumed overall (Balentine, Wiseman, & Bouwens, 1997; da Silva Pinto, 2013; Popkin, Bray, Caballero, Frei, & Willett, 2009). These non-herbal teas come from the *Camellia sinensis* L. plant. Other tea varieties include white, yellow and Pu er (or Pu-erh) teas, and they differ from each other in appearance, color, organoleptic taste, flavor, aroma and chemical content due to processing and aging (Balentine, Wiseman, & Bouwens, 1997; Gascoyne, Marchand, Desharnais, & Américi, 2014; Heck & Gonzalez de Mejia, 2009; Sharangi, 2009).

Herbal tea is an infusion that is brewed from the leaves, flowers, seeds, fruits, nuts, stems or roots of single or multiple plant species rather than *Camellia sinensis* (Lasekan & Lasekan, 2012; Y. Liu, Ahmed, & Long, 2013). In general, herbal tea infusions are prepared following a few simple steps. First, boiling water is poured over the plant material and they are stirred. Then, the mixture is steeped for a couple minutes and filtered to separate the aqueous extract (Tschiggerl & Bucar, 2012).

Herbal teas, also referenced as tisanes, have been consumed worldwide for pleasure, health care and disease prevention (da Silva Pinto, 2013; Heck & Gonzalez de Mejia, 2009; Lasekan & Lasekan, 2012; Wong, Liang, Chen, & Zhao, 2015). Rooibos (*Aspalathus linearis*), rose hip (*Rosa* spp.), chamomile (*Matricaria recutita*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare* subsp. *vulgare*), lavender (*Lavandula angustifolia*), thyme (*Thymus*

*vulgaris*), maté (*Ilex paraguariensis*), hibiscus (*Hibiscus sabdariffa*), and peppermint (*Mentha piperita*) are just some examples of the many herbal teas consumed around the world (da Silva Pinto, 2013; Heck & Gonzalez de Mejia, 2009; Tschiggerl & Bucar, 2012).

Most teas are consumed in their production countries or regions and the global tea supply chain is mostly controlled by few multinational companies (CBI, 2015). The grade of tea is determined by its quality attributes (aroma, taste, color, conditions of the leaves), and it affects tea market prices, which can vary from a few to hundreds of dollars per kilogram (Kraujalytė, Pelvan, & Alasalvar, 2016; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013)

The use of native wild plants for making medicinal tea has been the main health care for the Chinese people for millennia (Hu, 1997). Herbal teas are known in Southern China as "*Liangcha*", which means "cooling tea" (translation of Chinese ideograms "*liang*" = cooling/cool and "*cha*" = tea) (Hu, 1997; Y. Liu, Ahmed, & Long, 2013; Wong, Liang, Chen, & Zhao, 2015). *Liangcha* are traditionally brewed from fresh or dried wild plants from the local environment and served warm, cold or at room temperature (Hu, 1997; Y. Liu, Ahmed, & Long, 2013).

Teas and herbal teas are part of Chinese history, economy, culture and medicine. According to Hu (1997), Southern China was historically considered the land of exile for degraded officials of the Chinese government. Due to the extreme weather conditions (cold dry winter and hot humid summer) of this mountainous area, ancient people used to suffer from several viral and infectious diseases which were thought of as plagues or deviltries (Hu, 1997).

The development of "*liangcha*" is resultant of Southern Chinese people's need for healthcare and prevention of diseases in an economical and convenient way (Hu, 1997; Y. Liu, Ahmed, & Long, 2013). Another type of herbal tea commercialized in China is named

"*medicated teas*", and they are parceled mixtures of traditional Chinese crude drugs that may or may not contain tea (*Camellia sinensis*) (Hu, 1997).

The global tea market is a fast growing consumer market and the increasing consumers' desire for accessible health tea and tea-based products has driven the tea industry to improve tea quality and safety (Heck & Gonzalez de Mejia, 2009). A recent marketing research conducted by Mintel (2015) confirms this fast growth of tea and the ready-to-drink (RTD) tea category in the United States. From 2010 to 2014, the sales in this market segment grew 20.6%, reaching $6.7 billion, with a sales growth of 19.7%, on track to reach $8.5 billion by 2020 (Mintel, 2015).

Several Chinese commercial herbal teas are composed of a mix of different herbs that are not always fully listed on their packages, which makes their analysis for safety and quality purposes even hard (Hu, 1997; Y. Liu, Ahmed, & Long, 2013; Long, Cui, Wang, Zhang, Zhang, Li, et al., 2014; Wong, Liang, Chen, & Zhao, 2015).

DNA barcoding can be an effective and viable technique to identify herbal teas and their possible adulterants (Long, et al., 2014), but other methods have been suggested as well. For example, in a recent study conducted in China, Wong, Liang, Chen, and Zhao (2015) used a microscopic technique and ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS/MS) to identify commercial *Xihuangcao* herbal tea bags containing multiple herbs. This study showed vine tea (*Ampelopsis grossedentata*) was found as one of the most common components in the samples, even though it was not always listed on the ingredients list (Wong, Liang, Chen, & Zhao, 2015). This illustrates the interest in vine tea products in China.

## 2.2 Vine tea (*Ampelopsis grossedentata*)

Vine tea is a herbal tea brewed from leaves and stems of the plant *Ampelopsis grossedentata* (Figure 1), which has been consumed in South China and Southeast Asia for many years, and traditionally has been used in herbal medicine (Du, Cai, Xia, & Ito, 2002; Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009; X. J. Zheng, et al., 2014).

Figure 1. Dried leaves and stems of commercial vine tea (*Ampelopsis grossedentata*).

*Ampelopsis grossedentata* (Hand.-Mazz.) W. T. Wang (显齿蛇葡萄) is the full botanic name of vine tea, which is also known as “Teng Cha” (or “Tengcha”), “Tocha”, “Rattan tea”, “Duan Wu Cha”, “Mao Yan Mei”, or “Moyeam” (Fang, Wang, & Tang, 2011; Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009; Kou & Chen, 2012; B. Liu, Du, Zeng, Chen, & Niu, 2009; Murakami, Miyakoshi, Araho, Mizutani, Kambara, Ikeda, et al., 2004; Wong, Liang, Chen, & Zhao, 2015; Ye, Wang, Duncan, Eigel, & O’Keefe, 2015; Zhao, Deng, Chen, & Li, 2013).

As shown in Figure 2, this “perennial woody vine plant” of the family Vitaceae, genus Ampelopsis, grows wild in the mountains and forests of Southern China (Fang, Wang, & Tang, 2011; Xia, Hu, Xiong, Li, Wan, & Lin, 2011; Ye, Wang, Duncan, Eigel, & O’Keefe, 2015).

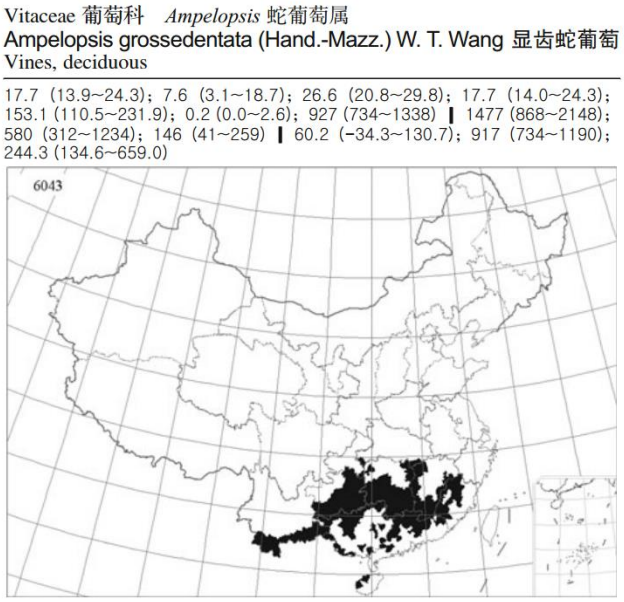


Figure 2. *Ampelopsis grossedentata*: botanical information, Chinese name, life form, and distribution in China. (Reprinted with permission from Frang, Wang, & Tang, 2011).

2.3 Chemical components in vine tea

Dihydromyricetin (Figure 3), also known as ampelopsin, is the major flavonoid component present in vine tea and also its most active component (Du, Cai, Xia, & Ito, 2002; Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009; Ying, Xu, Huang, & Wang, 2011; X. J. Zheng, et al., 2014). However, several other flavonoids were reported to be found in leaves of *Ampelopsis grossedentata*, including myricetin, vitexin, quercitrin, luteolin, quercetin, apigenin, kaempferol and rutin (Ying, Xu, Huang, & Wang, 2011).

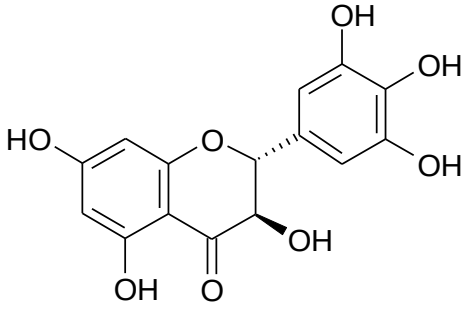


Figure 3. Chemical structure of dihydromyricetin (ampelopsin).

Dihydromyricetin ($C_{15}H_{12}O_8$) and myricetin ($C_{21}H_{20}O_{12}$) are both considered strong antioxidants because they are flavonoids with six hydroxyl groups (Zhong, Kong, Zhou, Zhou, Zhang, & Wang, 2014). The presence of dihydromyricetin and myricetin in naturally fermented vine tea was also reported by X. J. Zheng, et al. (2014), who identified three other flavonoids: 3-dihydroxyquercetin, iso-dihydromyricetin and myricetin-3-rahmnose.

Even though leaves of *Ampelopsis grossedentata* contains high amount of dihydromyricetin, it is hard to obtain this flavonoid component with high purity (Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009). Dihydromyricetin has poor solubility properties in both aqueous and non-aqueous systems (Li, Wu, Liu, Hou, Wan, Lou, et al., 2015). Du, Cai, Xia, and Ito (2002) obtained (+)-dihydromyricetin at high purity (over 99%) from dried leaves of *Ampelopsis grossedentata* using a high-speed counter-current chromatograph (HSCCC) equipped with three scale-up columns for purification. In further study, HSCCC was used to obtain two flavonoid glycosides from leaves extract of *Ampelopsis grossedentata*: 5,7-dihydroxy-3',4'-trihydroxyflavone-3-O-6"-rhamnose and 5,7-dihydroxy-3',4'-dihydroxyflavone-3-O-6"-rhamnose (Du, Chen, Jerz, & Winterhalter, 2004). However, the HSCCC process is not simple for industrial use, so Gao, Liu, Ning, Zhao, Zhang, and Wu (2009) suggested that the simpler process of recrystallizing from water five separate times could produce dihydromyricetin about 95% pure.

Microwave multi-stage countercurrent extraction (MMCE) was suggested by Li, Zheng, Wang, Shao, Gao, Ning, et al. (2007) as an alternative method in terms of cost and efficiency for the extraction of dihydromyricetin from vine tea leaves. This method was reported as 20-30% more efficient than microwave static batch extraction (MSBE) under similar conditions (Li, et al., 2007).

In addition to the group of flavonoids, D.-Y. Wang, Zheng, Xu, and Zheng (2002) isolated four isoflavones from the *n*-BuOH fraction of the MeOH extract of *Ampelopsis grossedentata*: 6,7-dihydroxy-3'-methoxy-4',5'-methylenedioxy-isoflavone; 6,7-dihydroxy-3'-methoxy-4',5'-methylenedioxyisoflavone 6-*O*-β-D-glucopyranoside; 6,7-dihydroxy-3'-methoxy-4',5'-methylenedioxyisoflavone 6-*O*-α-L-rhamnopyranoside; and 6,7-dihydroxy-3'-methoxy-4',5'-methylenedioxyisoflavone 6-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside.

In their previous study, D. Wang, Liu, Lu, and Zheng (1999) isolated six limonoids from the EtOAc fraction obtained by partition of the MeOH extract of *Ampelopsis grossedentata*: rutaevin-7-*O*-gallate, rutaevin-7-*O*-caffeate, rutaevin acetate, nomilin, rutaevin, and methyl deacetylnomilinate. An important aspect of limonoids is they are chemical compounds of moderate polarity and typically bitter in taste (Roy, Amit, & Shailendra, 2006)

Fatty acids are another important groups of chemical components present in vine tea. Fan, Chen, Chen, and Suo (2014) identified thirty-one free fatty acids were identified in vine tea. Their study showed 18:2ω6 (linoleic acid), 18:0 (octadecanoic, stearic), 16:0 (hexadecanoic, palmitic), 18:3ω3 (linolenic), 18:3ω6 (α-linolenic acid) and 20:4ω6 (arachidonic acid) were the major fatty acids present in the vine tea. Arachidonic is particularly noteworthy and, if validated by additional study, would be quite interesting and unusual. Linoleic acid was reported as the primary unsaturated fatty acid (PUFA), and octadecanoic acid was identified as the primary saturated fatty acid (SFA) in vine tea (Fan, Chen, Chen, & Suo, 2014).

Besides their nutrient value, fatty acids also contribute to aroma. Even though volatile components are of great importance to the quality of herbal teas, no information about flavor chemistry and aroma profile of vine tea was found in current literature. In their paper, Fan, Chen,

Chen, and Suo (2014) reported the amount of fatty acids was affected by harvest time (April, July or September), which suggests vine tea aroma may be similarly affected.

2.4 Vine tea's properties and potential applications

*Ampelopsis grossedentata* has been a significant plant resource in medicinal food research (Zheng et al., 2014). Kou and Chen (2012) reviewed the pharmacological potential of dihydromyricetin and vine tea has gained increasing attention over the last years due to ampelopsin's wide range of biological functions and interest in the products.

Physical performance under simulated high altitude was increased by dihydromyricetin, and its use may possibly avoid exercise intolerance and altitude-related illnesses credited to hypobaric hypoxia (Zou, Chen, Liu, Chang, Zhu, & Mi, 2014).

Dihydromyricetin extracted from vine tea has presented significant anti-inflammatory properties *in vitro* and *in vivo*, and it has been recommended as a potential therapeutic agent for inflammatory-related diseases (Chen, Zhao, Wan, Ran, Qin, Wang, et al., 2015; Hou, Tong, Wang, Shi, Xiong, Chen, et al., 2015; Qi, Xin, Guo, Diao, Kou, Luo, et al., 2012).

Qi, et al. (2012) suggested the anti-inflammatory effect of dihydromyricetin is attributable to inhibiting the interconnected reactive oxygen species (ROS)/Akt/IκB kinase (IKK)/nuclear factor κB (NF-κB) signaling pathways. A study reported that dihydromyricetin supplementation in patients with nonalcoholic fatty liver disease can improve their lipid, glucose and liver enzymes metabolism, increase insulin resistance, protects liver function, and reduces the progression of liver steatosis (Chen, et al., 2015).

The anticarcinogenic properties of vine tea have been investigated in the past few years and studies have suggested dihydromyricetin is a potential carcinopreventive agent. Dihydromyricetin inhibited the migration and invasion of hepatoma cells and may prevent

hepatocellular carcinoma (HCC) metastasis (Zhang, Li, Zeng, Liu, Liu, Shu, et al., 2014). Moreover, ampelopsin showed a protective effect of neuronal-like PC12 cells against $H_2O_2$-induced cytotoxicity, which suggests it can be potentially used for treatment of neurodegenerative diseases as well (Kou, Shen, An, Qi, Dai, & Yin, 2012).

The antioxidant properties of vine tea's main flavonoid dihydromyricetin have also been objective of study in the past years (Baek, Neilson, Eigel, & O'Keefe, 2015; Xin, Ma, Lin, Xu, & Chen, 2015; Xin, Ma, Xu, & Chen, 2013a, 2013b; Ye, Wang, Duncan, Eigel, & O'Keefe, 2015; Y. Zhang, Ning, Yang, & Wu, 2003; Q. Zheng, Xu, Zhu, Chen, Liu, Chen, et al., 2010). Increasing shelf life by reducing oxidative deterioration in foods is of great value to the food industry, and the use of natural antioxidants attends consumer's desire of clean labels (Baek, Neilson, Eigel, & O'Keefe, 2015). Zhang, Ning, Yang, and Wu (2003) reported dihydromyricetin in the concentration range of 0.01% - 0.04% was effective in preventing the increase of lipid peroxidation values in a linoleic acid system catalyzed by $FeSO_4$-edetic acid. Dihydromyricetin-rich extract from vine tea improved stability of tocopherol stabilized menhaden oil, especially when used combined with rosemary extract (Baek, Neilson, Eigel, & O'Keefe, 2015).

Ye, Wang, Duncan, Eigel, and O'Keefe (2015) analyzed the antioxidant activities of both dihydromyricetin and vine tea extract and compared with synthetic antioxidant butylated hydroxyanisole (BHA) in cooked ground beef and soybean oil. Vine tea extract, dihydromyricetin, and BHA all had similar antioxidant effect in cooked ground beef. Dihydromyricetin was more efficient than BHA in inhibiting soybean oil oxidation, but vine tea extract was not successful at inhibiting the formation of secondary oxidation products. (Ye, Wang, Duncan, Eigel, & O'Keefe, 2015).

Researchers have emphasized dihydromyricetin is a potential ingredient for functional food and nutraceuticals, however its poor solubility and lipophilicity properties is a limiting to its processability and application in food industry (Li, et al., 2015; B. Liu, Du, Zeng, Chen, & Niu, 2009).

A dihydromyricetin–lecithin complex was suggested by Liu, Du, Zeng, Chen, and Niu (2009) to improve its solubility in oil. Results showed the complex significantly improved the solubility of dihydromyricetin in *n*-octanol, but the stability of the complex in oil was worse when the ratio of lecithin and dihydromyricetin was lower than 1. Moreover, the dihydromyricetin–lecithin complex was an efficient scavger of DPPH radicals, more active than butylated hydroxytoluene (BHT) (Liu, Du, Zeng, Chen, & Niu, 2009).

The antibrowning effect of dihydromyricetin, isolated from the pine needles of *Cedrus deodara*, was analyzed by Liang, Wu, Qiu, Zhong, and Gao (2014). Their study showed a low concentration of dihydromyricetin (0.05%) was effective in inhibiting browning of fresh-cut apple slices and this potential antibrowning agent combined with ascorbic acid resulted in a synergistic effect (Liang, Wu, Qiu, Zhong, & Gao, 2014). These authors also reported the need of further sensory and toxicological evaluation of dihydromyricetin. Studies reporting antibrowning properties of vine tea extracts were not found.

It is well known that packaging materials contribute to reduction of food degradation and increased shelf-life. Some smart packages made with anti-oxidants can increase shelf-life and sensory and nutritive quality of food by reducing its oxidation process (Duncan & Webster, 2009).

A study of the thermooxidative stability of polypropylene (PP), linear low density polyethylene (LLDPE), high density polyethylene (HDPE), polystyrene (PS), ethylene vinyl

acetate copolymer (EVA), natural rubber (NR), and nitrile butadiene rubber (NBR) stabilized by dihydromyricetin showed dihydromyricetin was more efficient than the synthetic antioxidant Irganox 1010 (3-({3-[4-Hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoyl}oxy)-2,2-bis[({3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl]propanoyl}oxy)methyl]propyl 3-[4-hydroxy-3,5-bis(2-methyl-2-propanyl)phenyl ]propanoate) as a thermally stable antioxidant for polymers (Zheng, et al., 2010).

Further studies also suggested dihydromyricetin could be an effective high value-added additive for use in polypropylene packaging material (Xin, Ma, Lin, Xu, & Chen, 2015), and an efficient unhindered phenolic antioxidant for LLDPE (linear low density polyethylene) stabilization (Xin, Ma, Xu, & Chen, 2013a). Additionally, Xin, Ma, Xu, and Chen (2013b) reported the antioxidant activity of dihydromyricetin in EVA (ethylene vinyl acetate) depends on pH and it loses stabilization functions under alkaline conditions.

2.5 Flavor of teas and herbal teas

Heath and Reineccius (1986) defined flavor as “a very complex sensation primarily composed of aroma and taste but also complemented by tactile and temperature responses”. Aroma and taste are detected in the nose and in the mouth respectively. The smell of food before it is put into the mouth is defined by the word “aroma”, while “odor” means the smell of food inside the mouth (Teranishi & Kint, 1993).

Even though taste and tongue’s reactions to tactile and temperature stimuli also contribute to overall flavor perception, aroma is the most important characteristic of flavor (Heath & Reineccius, 1986). According to Chaturvedula and Prakash (2011), aroma is a critical quality attribute of tea which can decisively impact consumer’s acceptability.

Tea odor and aroma are a result of volatile compounds, while color and the taste are mostly a consequence of phenolic compounds (Yang, Baldermann, & Watanabe, 2013). Instrumental techniques and sensory procedures are important tools that can be applied to analyze quality attributes of tea (Qin, Pang, Chen, Cheng, Hu, & Wu, 2013).

It is known that flavor affects herbal teas acceptability, and "robust", "refreshing", and "tea-like" characteristic flavors are greatly desired in high quality herbal teas (Lasekan & Lasekan, 2012). The complex set of volatile components present in tea and tea products includes aldehydes, alcohols, and lactones (Yang, Baldermann, & Watanabe, 2013). For example, $C_6$ alcohols and aldehydes contribute to the typical "green", "grassy", "fresh" flavor notes present in some teas (Cheetham, 2002). Flavor and aroma volatile compounds are present in low concentrations, parts per million (ppm) or lower, in foods (Teranishi & Kint, 1993). Each food or beverage contains a great amount of different flavors chemicals, and each has specific taste characteristics and aroma thresholds. A molecule is an active component of an aroma when its concentration is higher than its threshold (Cheetham, 2002). The flavor of a food or beverage is a dynamic equilibrium that can change as result of many factors (Heath & Reineccius, 1986). Temperature, pH and other environmental conditions affects the perceived quality and intensity of a flavor (Cheetham, 2002).

Processing conditions and type of tea leaf used also have a great influence on tea flavor and aroma (Cheetham, 2002). As reported by Sheibani, Duncan, Kuhn, Dietrich, Newkirk, and O'Keefe (2015), panning (a common step in tea manufacturing) formed new aroma active compounds and affected flavor of oolong tea by reducing significantly the amount of alcohols, ketones, acids and esters and increasing the amounts of linalool oxide, *cis* jasmone, and methyl salicylate.

## 2.6 Analysis of volatile compounds

The identification of volatile flavor components in food is very challenging for several reasons. First, even today laboratory equipment is not as sensitive as the human olfactory system for detection of many odors (relative sensitivity is variable). Second, food flavor is dispersed in a food matrix that may contain water, carbohydrates, proteins and lipids, all of which can affect the flavor extraction process. Third, flavors consist of a large number of chemicals classes, which may include acids, alcohols, aldehydes, amines, ketones, aromatics, and heterocyclics (Heath & Reineccius, 1986). The differences in polarity and volatility make recovery of aroma volatiles as a single extract challenging.

After the flavor components are extracted and identified, a significant question to be answered is related to the importance of each chemical to the overall flavor of the specific food or beverage (Heath & Reineccius, 1986). Some molecules have very low odor detection thresholds, so even in small quantities they contribute significantly to the overall flavor of foods and beverages. Also, isomers of a molecule may contribute with different flavor characteristics and intensities (Cheetham, 2002).

Several solvent extraction and distillation techniques can be applied to extracting volatile components from food samples (Lasekan & Lasekan, 2012). Direct organic solvent extraction, simultaneous distillation and extraction (SDE), steam distillation under reduced pressure (SDR), brewed extraction, adsorptive column method, stir bar sorptive extraction (SBSE), solvent-assisted flavor evaporation (SAFE), solid phase microextraction (SPME), and dynamic headspace sampling (DHS) are some techniques that may be used to extract tea volatile compounds (Yang, Baldermann, & Watanabe, 2013).

Brewed extraction is a simple sample preparation method that reproduces very well the volatile composition of the tea sample and minimally affects its aroma components (Yang, Baldermann, & Watanabe, 2013). In this method, boiling water is used to brew a tea sample for a determined period of time and the infusion is filtered (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995). As water is the main solvent used for tea preparation and extraction, most compounds that can be extracted by infusion are of high polarity (Tschiggerl & Bucar, 2012).

The next step to analyze volatile components is the separation of volatiles from their aqueous solution (Tschiggerl & Bucar, 2012). Headspace methods, often combined with trapping technologies, are commonly used to collect volatiles from food samples (Lasekan & Lasekan, 2012). Solid phase microextraction (SPME) is a simple, effective and solvent-free method to collect volatiles where fused silica fibers coated with different types of polar or nonpolar adsorbents are used to adsorb and desorb volatiles from the samples (Alpendurada, 2000; Kataoka, Lord, & Pawliszyn, 2000; Yang, Baldermann, & Watanabe, 2013).

In this extraction process the fiber is exposed to the sample matrix or to its headspace for a determined period of time (Alpendurada, 2000; Lord & Pawliszyn, 2000). Usually, the fused silica fiber is part of a stainless steel needle which is fitted together with an appropriated device (Figure 4) (Rubiolo, Sgorbini, Liberto, Cordero, & Bicchi, 2010).

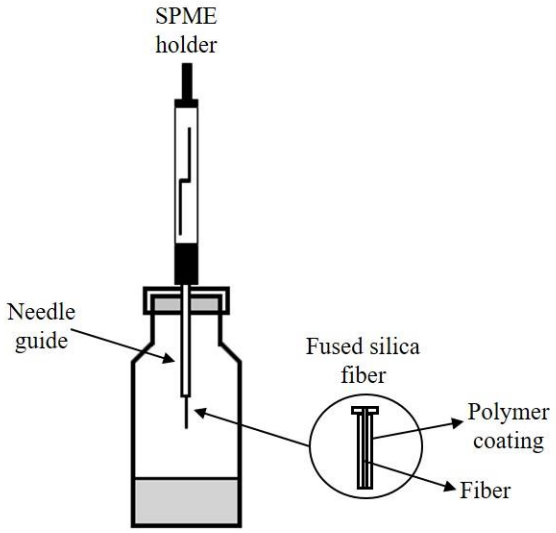


Figure 4. Diagram of headspace sampling using SPME. Reproduced from Rubiolo, Sgorbini, Liberto, Cordero & Bicchi, 2010.

Nonpolar fiber coatings, for example polydimethylsiloxane (PDMS), are used to extract nonpolar volatile and semivolatile compounds, and polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibers can be used to extract more polar volatiles (Kataoka, Lord, & Pawliszyn, 2000; Yang, Baldermann, & Watanabe, 2013). Both PDMS and DVB chemical structures are shown in Figure 5.

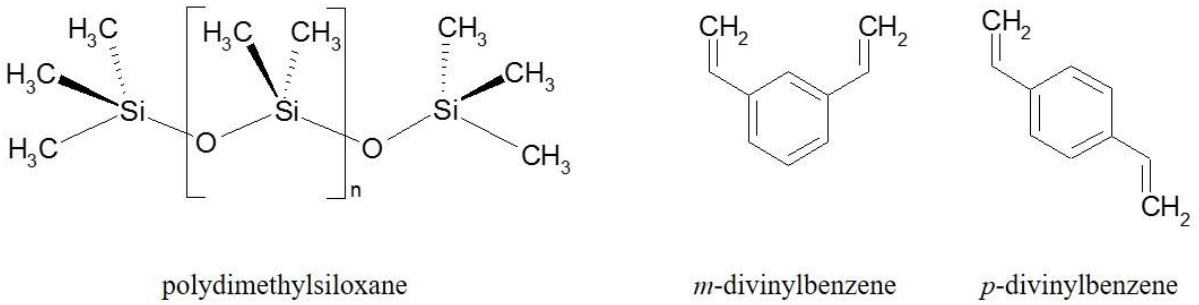


Figure 5. PDMS and DVB chemical structures.

SPME can be used to gather volatiles from dry tea or tea infusion samples, and the efficiency of this method depends on sampling conditions such as volume, time, and temperature (Yang, Baldermann, & Watanabe, 2013).

Gas chromatography (GC) was invented in the nineteen-fifties and its development and improvement has led to the current information about flavor chemistry (Ohloff, Flament, & Pickenhagen, 1985). Today, GC is the most common method to analyze composition of isolated volatile fractions and it is normally used together with detection methods such as mass spectrometry, flame ionization detection, or olfactometric detection (Tschiggerl & Bucar, 2012). As shown in Figure 6, a GC is composed by an injector, a capillary column located inside an oven, and a detector (Bouchonnet, 2013).

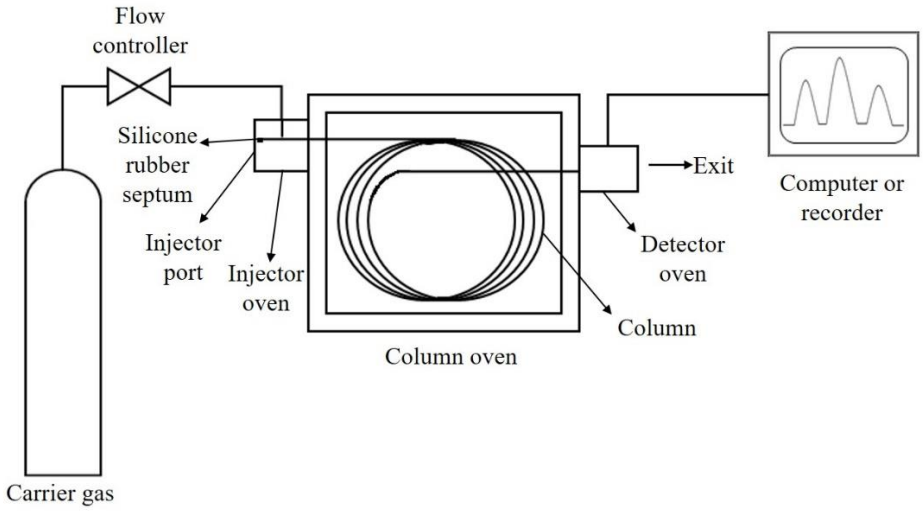


Figure 6. Diagram of a gas chromatograph.

Hydrogen, nitrogen and helium are typically used as carrier gases in GC analysis. Both hydrogen and nitrogen are cheap options available, but hydrogen is explosive and nitrogen has high viscosity, which makes it an inefficient carrier gas. Helium is inert and has low viscosity, so even though it is an expensive option it is the most used carrier gas. (McMaster, 2008).

The sample injection process can be manual or automated, and the automated process is greatly recommended when there are multiple samples to be analyzed. In this case, the GC needs to be equipped with an autosampler, which is a robotic arm that takes a sample vial from a tray or carousel and then injects the sample into the GC (McMaster, 2008). Some common types of injections are split mode, splitless mode and on-column injection (Bouchonnet, 2013).

The use of capillary column GC has become usual practice to work with flavor chemistry, and it provides highly reproducible retention times, which is very important for identification of volatiles based on Kovats or Linear Retention Index (LIR) and use of computerized pattern recognition (Heath & Reineccius, 1986).

Based on the stationary phase, the three main types of capillary columns are apolar, low polarity (or semi- polar), and polar. In general, apolar columns contain a silica film with methyl groups, and for the low polarity column some methyl groups are replaced by phenyl groups. Polar columns have polar components in their stationary phase, for example polyethylene glycols or cyano groups (Bouchonnet, 2013).

Retention time is a characteristic of each volatile component and does not change under constant GC conditions. However, different compounds can have the same retention time, which may lead to ambiguous identification (Mussinan, 1993).

The retention time of an unknown volatile component can be determined in relation to the retention time of standard chemical compounds. In 1958 Kovats developed a system that uses *n*-paraffins as standards, which have an index equal to a hundred times the number of carbon atoms, and it became the most common system used in flavor analysis (Mussinan, 1993).

Mass spectrometry is the second most used technique to analyze volatile components and usually it is used as a mass-selective GC detector or to identify an unknown compound

(McMaster, 2008; Reineccius, 2002). In the most typical ionization process, electron impact (EI), a stream of electrons is used to break the chemical bonds and ionize the sample components. In sequence, a mass analyzer takes the accelerated fragment ions and separates them according to their mass-to-charge ratio (m/z). Lastly, a bar graph will be generated representing the mass spectrum of the unknown component and it can be compared to a library of known spectra for identification (Mussinan, 1993).

However, gas chromatography-mass spectrometry (GC-MS) alone does not provide an odor description of the identified volatile components neither information about their significance to the overall aroma and flavor of the food. Gas chromatography-olfactometry (GC-O) is a technique used in flavor analysis that allows a trained person to help the detection and evaluation of volatile components eluting from GC separation (Reineccius, 2002). Also, sensory methods such as descriptive analysis are commonly applied to characterize aroma and flavor of foods.

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# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Samples

Vine tea samples (100% vine tea) were purchased in replicate packages from tea stores in China. Three brands were randomly chosen for analysis. Brand A samples had dried leaves and stems of *Ampelopsis grossedentata* packed in a resealable opaque plastic bag. They were purchased in summer of 2010 and stored at room temperature in the dark in original, unopened packaging. Brand B had vine tea dried leaves and stems packed in a non resealable opaque plastic bag which was inside a cardboard carton package. Brand C had ground dried leaves and stems of vine tea packed in tea bags, which were inside cardboard carton packages. Both brands B and C samples were purchased in January, 2016.

## 3.2 Vine tea infusion preparation

Distilled water (DI) and Blacksburg (VA, USA) tap water were both used to prepare vine tea infusions. As described by Sheibani (2014), Blacksburg tap water profile is: chloride = 15 ppm, sodium = 10 ppm, calcium = 8 ppm, magnesium = 6 ppm, potassium = 5 ppm, sulfate = 2 ppm, carbonate = 6 ppm, bicarbonate = 47 ppm, total alkalinity (reported as $CaCO_3$) = 49 ppm, total hardness (reported as $CaCO_3$) = 45 ppm, and total dissolved solids = 100 ppm.

Boiling water (98°C) was poured over vine tea dried leaves and stems in a ratio of 2g of tea per 100 mL of water, and mixtures were brewed for 5 minutes. Then, the infusions were filtered using gravity filtration technique and Fisherbrand™ qualitative grade plain filter paper circles, P4 grade paper, diameter 24.0 cm, medium-fine porosity, and slow flow rate (distributed by Fisher Scientific, Pittsburgh, PA, USA, made in U.K.). All laboratory glassware was washed, rinsed with DI water and baked overnight at 160°C before use.

3.3 Effects of water quality on pH, color and volatile profiles of vine tea infusions

Two factor experiments were performed in triplicate to investigate the effects of water quality (distilled water vs. tap water), vine tea sort (brands A, B, and C), and interaction on pH, color and volatile profiles of vine tea infusions. Results were analyzed using analysis of variance (ANOVA) and mean comparisons made using Tukey's HSD test with 5% significance level ($\alpha$=0.05). Statistical analyzes were performed using JMP® Pro 11.0.0 statistical analysis software (SAS, Cary, NC, USA).

3.4 pH measurement

The pH values of vine tea infusions were measured using a Fisher Scientific™ Accumet™ Research AR25 pH/mV/°C/ISE Meter (Fisher Scientific, Pittsburgh, PA, US). Buffer solutions (pH 4.0 and pH 7.00) (Fisher Scientific, Pittsburgh, PA, US) were used to calibrate the equipment. Vine tea infusion samples were measured in triplicate.

3.5 Color measurement

A Minolta CR-300 Chroma Meter (Minolta Co., Osaka, Japan) was used to measure the reflective color of vine tea infusions in CIE L*a*b* (CIELAB) system. A standard white plate (L* = 96.77, a* = 0.45, b* = 2.37) was used do calibrate the equipment and in sequence L*a*b* values for vine tea infusions were recorded. L* values range from 0 (darkness, black) to 100 (lightness, white). Positive and negative a* values represent redness and greenness, respectively. Positive b* values represent yellowness and negative values represent blueness. Vine tea infusions (30 mL) were pipetted into glass test tubes which were placed in a special support for liquids (CRA-70, Minolta Co., Osaka, Japan). Samples were measured in triplicate.

3.6 Volatile extraction by SPME and GC-MS analysis

Volatile compounds of vine tea infusions were extracted by headspace solid-phase micro-extraction (HS-SPME) and analyzed with gas chromatography-mass spectrometry (GC-MS) using the method adapted from Sheibani, Duncan, Kuhn, Dietrich, Newkirk, and O'Keefe (2015).

Volatile headspace compounds from vine tea infusions were identified by using a Shimadzu GCMS-QP2010 Ultra equipped with an AOC-5000 Plus SPME auto-sampler (Shimadzu Scientific, Columbia, MD, USA), which was used for sample extraction and injection into the GC-MS.

Vine tea samples were equilibrated for 2 minutes prior to extraction and in sequence a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 µm, 2 cm length) (Supelco, Bellefonte, PA, USA) was exposed to the headspace above vine tea infusions in amber glass vials for 30 min at 40°C with an agitation speed of 250 rpm.

Volatiles desorbed from the fiber were separated by using a polyethyleneglycol capillary column (ZB-Wax plus; 60 m × 0.25 mm i.d. × 0.25 µm film thickness; Phenomenex, Torrance, CA, USA). A Shimadzu GCMS- QP2010 Ultra gas chromatograph with mass selective detector (Shimadzu Scientific, Columbia, MD, USA) were used to analyze the volatile compounds of vine tea.

Initial oven temperature was held at 40°C for 0.5 min and then increased to a final temperature of 240°C with a temperature ramp rate of 8º C/min. Injections were made in splitless mode with injector temperature of 240°C. Ultra-high purity helium was used as the carrier gas at a flow rate of 0.5 mL/min and linear velocity of 30 cm/sec. The mass spectra were performed every 0.3 sec and data collected from m/z 40 – 400. The ion source and quadrupole were set to 230 and 200°C, respectively.

3.7 Effect of salt on the extraction of vine tea volatile components

In this study, the effect of adding salt (NaCl) to vine tea infusions on the extraction of volatile components was investigated. Different vine tea samples were used in each replication (brands A, B, and C), but only the effect of salt addition on the area of volatile component peaks was analyzed.

First, 2.0 g of salt (NaCl) were added to half of the 20 mL amber glass headspace vials (Supelco, Bellefonte, PA, USA). Then, 10 mL of filtered vine tea infusions prepared with distilled water were pipetted into the vials, which were closed with magnetic screw caps with18 mm thread with PTFE/silicone septum (white PTFE/transparent blue silicone), septum thickness 1.3 mm (Supelco, Bellefonte, PA, USA). All samples with salt added were manually agitated for few seconds before analysis. Clean glassware and vials were rinsed with DI water and baked overnight at 160°C before use.

In sequence, samples were analyzed in triplicate using HS-SPME and GC-MS methods previously described. Shimadzu software was used to plot chromatograms and the NIST 11 (Scientific Instrument Services, Ringoes, NJ, USA) and Wiley (John Wiley and Sons Inc.) libraries were used as references to identify volatile compounds in vine tea by similarity to fragmentation spectra of standards. The peak areas of only vine tea volatile components identified in all the samples were compared using paired t-test with 5% significance level. Statistical analysis was performed using JMP® Pro 11.0.0 (SAS, Cary, NC, USA).

3.8 Identification of volatile components present in vine tea infusions

Vine tea volatile characterization through HS-SPME and GC-MS took place at the Food Analysis Laboratory located in the Food Science and Technology Building (FST) (Virginia Tech, Blacksburg, VA, USA) from February to March 2016.

Filtered vine tea infusion (10 mL) and 2.0 g of salt (NaCl) were placed into 20 mL amber glass headspace vials (Supelco, Bellefonte, PA, USA) with 18 mm magnetic screw thread cap with PTFE/Red Chlorobutyl or PTFE/Silicone septa (Restek, Bellefonte, PA, USA). Samples were kept refrigerated at 4°C until the GC-MS analyzes were performed. All glass vials were new and prepared by rinsed with distilled water and baked overnight at 160°C before use. Glass vials and screw caps were not reused.

HS-SPME and GC-MS analysis were conducted following techniques described earlier. Chromatograms were plotted by Shimadzu software and volatile components were first identified by comparison of their mass spectra to fragmentation spectra of standards of the NIST 11 (Scientific Instrument Services, Ringoes, NJ, USA) and Wiley (John Wiley and Sons Inc.) libraries.

Additionally, a series of *n*-alkanes (C5, C6, C7, C8, C10, C12, C14, C16, C18, C20, C22, C24) (Supelco, Bellefonte, PA, USA) was used to calculate Kovats Indexes (also called LRI, Linear Retention Index) using linear regression. Then, online databases Flavornet (http://www.flavornet.org/flavornet.html) and Pherobase (http://www.pherobase.com/) were consulted for confirmative identification of volatile compounds by matching Kovats values for carbowax columns.

## REFERENCES

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Sheibani, E., Duncan, S. E., Kuhn, D. D., Dietrich, A. M., Newkirk, J. J., & O'Keefe, Sean F. (2015). Changes in flavor volatile composition of oolong tea after panning during tea processing. *Food Science & Nutrition*, n/a-n/a.

# CHAPTER 4

# RESULTS AND DISCUSSIONS

## 4.1 pH characterization of vine tea infusions

The pH values of vine tea infusions brewed with distilled (DI) water and Blacksburg (VA, USA) tap water (pH=6.38±0.03) are shown in Table 1.

Table 1. pH values of vine tea infusions

| Vine tea | Water quality | pH |
|---|---|---|
| A | Distilled water | 4.39±0.03[a] |
| | Tap water | 4.77±0.03[b] |
| B | Distilled water | 4.38±0.04[a] |
| | Tap water | 4.99±0.03[c] |
| C | Distilled water | 4.29±0.01[d] |
| | Tap water | 4.66±0.04[e] |

[a-e] Means ± SD followed by different letters are significantly different (P<0.05).

As expected, water quality had a significant effect on pH of vine tea infusions (P<0.05) and pH values were higher when tap water was used. Vine tea sample and interaction factor also affected pH values significantly (P<0.05). Brand A infusions had the highest pH values while brand C infusions had the lowest. The interaction factor between the two factors (water quality and vine tea sample) can be observed in the interaction profile plot below (Figure 7). The non-parallel lines in the plot are a good indication of interaction between the factors.

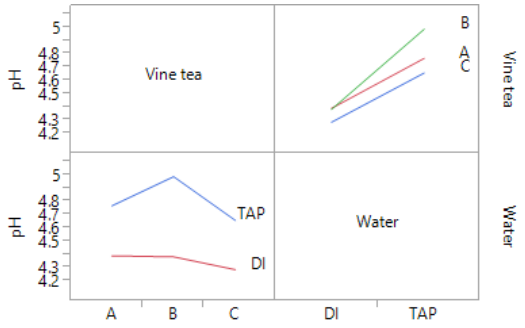


Figure 7. Interaction profile plot (pH)

Overall, vine tea infusions had acid pH values when prepared with both DI and tap waters: 4.53±0.06 and 4.80±0.14, respectively. The significant pH difference between samples is probably an effect of the ions present in Blacksburg tap water, which were relatively high in bicarbonate, which provides buffering capacity. The pH differences may also impact other characteristics of vine tea infusions, such as color, flavor and taste. Although Blacksburg water is considered soft, the high bicarbonate relative to total hardness most likely provides a buffering capacity that resulted in higher pH values compared to DI controls. Other differences include chloramine in Blacksburg water and Fluoride, but these are not likely to appreciably affect pH themselves.

Studies have shown that pH is an important parameter for optimization of the extraction of green tea constituents such as catechins, caffeine and theanine (Vuong, Golding, Stathopoulos, & Roach, 2013). As vine tea and its constituents also have great potential application in the food industry, similar studies of the impact of brewing solution pH are suggested to optimize the productions of vine tea extracts and powders.

4.2 Color characterization of vine tea infusions

Infusion color is an important quality attribute in the analysis of tea infusions (Chaturvedula & Prakash, 2011; Lin, Yang, Hsieh, Liu, & Mau, 2014). The CIE L*a*b* (CIELAB) color dimensions for vine tea infusions extracted with distilled water (L* = 33.59±0.51; a* = -0.05±0.16; b* = -0.35±0.03) and Blacksburg (VA, USA) tap water (L* = 33.36±0.44; a* = -0.16±0.14; b* = -0.27±0.04) are reported in Table 2:

Table 2. CIELAB color of vine tea infusions

| Vine tea | Water quality | L* | a* | b* |
|---|---|---|---|---|
| A | Distilled water | 26.45±0.47[a] | 0.87±0.19[a] | 6.56±0.16[a] |
| | Tap water | 25.14±0.29[b] | 1.29±0.10[a,c] | 5.21±0.23[b] |
| B | Distilled water | 28.51±0.76[c] | -0.13±0.39[b] | 8.62±0.25[c] |
| | Tap water | 26.35±0.93[a] | 1.40±0.23[c] | 7.13±1.03[a,b] |
| C | Distilled water | 26.56±0.61[a] | 1.45±0.20[c] | 7.82±0.69[d] |
| | Tap water | 25.75±0.38[a,b] | 2.00±0.25[d] | 6.91±0.45[a] |

[a-d] Means ± SD followed by the different letters in the same column are significantly different (P < 0.05).

Both vine tea type and water quality factors had significant effect in all dimension of CIELAB color space (P<0.05). The interaction factor between the two factors was also significant for L* and a* dimensions (P<0.05), but not for b* (P>0.05). Figure 8 shows the interaction profiles plots for all L*a*b* color responses. As can be observed, the lines are approximately parallel in the dimension b plot, which is a good indication of non-interaction between factors.

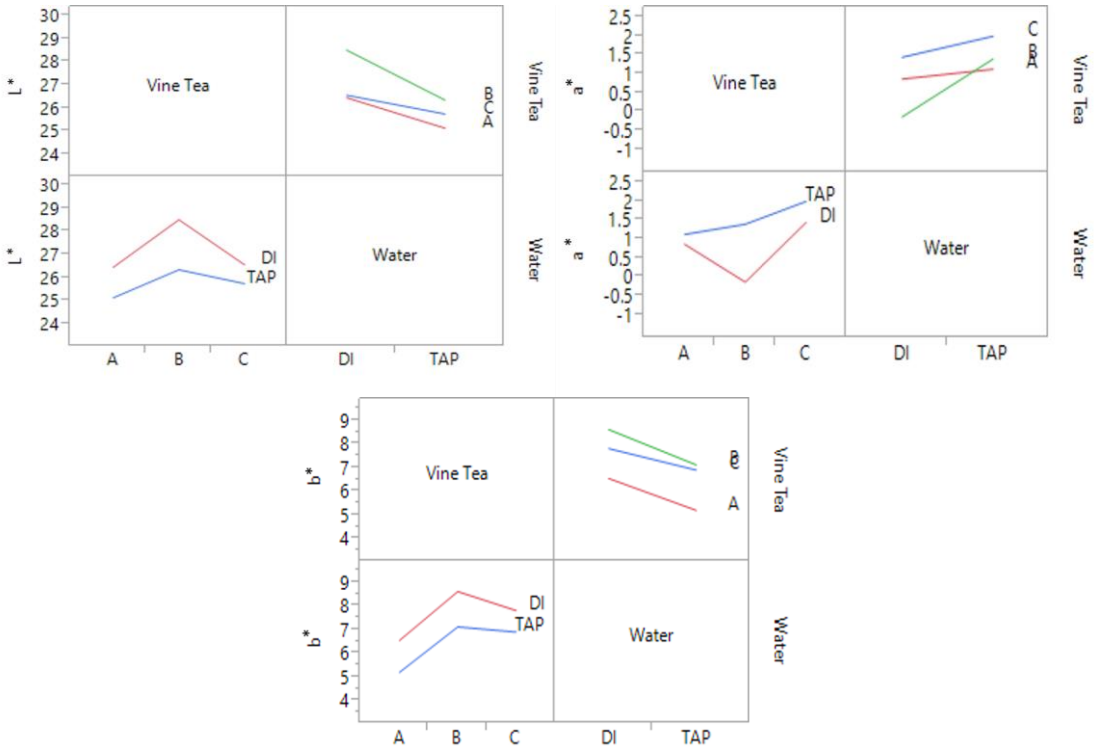


Figure 8. Interaction profile plots of CIELAB color dimensions

L* values range from 0 (darkness) to 100 (lightness). Positive and negative a* values represent redness and greenness, respectively. Positive b* values represent yellowness and negative values represent blueness. The low L*-values (DI=27.18±1.13; Tap=25.75±0.77) for vine tea infusions indicates darkness as a characteristic of their color. Also, the positive a*-values (DI=0.73±0.71; Tap=1.51±0.42) and the positive b*-values (DI=7.66±0.96; Tap=6.42±1.08) indicates redness and yellowness, respectively.

Overall, vine tea samples brewed with tap water were darker than the ones brewed with DI water. Studies have shown color of black and green tea drinks were adversely affected by water hardness (Kovalenko & Vietrov, 2015; Murugesan, Venkateswaran, & Manigandan, 2012), and black tea samples were darker when prepared with harder water than with distilled water (Murugesan, Venkateswaran, & Manigandan, 2012).

Flavonoids have a relationship with plant colors (Wang, Park, Chung, Baik, & Park, 2004). Vine tea is known to be rich in flavonoids, which possibly are the major contributors for its infusions typical color. However, the relationship between color and chemical components was not evaluated in this study (we were unable to investigate what specific components caused the color differences). So, further studies are suggested to investigate which chemical components are mostly responsible for vine tea infusions color. Sensory studies are also suggested for descriptive characterization of vine tea infusions color and a better understanding of consumer's preference and acceptability.

## 4.3 Effect of salt addition in the extraction of vine tea volatile components

Ten volatile components present in all samples were identified by similarity to fragmentation spectra of standards: β-cyclocitral ($C_{10}H_{16}O$), 1-penten-3-ol ($C_5H_{10}O$), (E,E)-2,4-heptadienal ($C_7H_{10}O$), (E)-2-hexenal ($C_6H_{10}O$), (E)-2-pentenal ($C_5H_8O$), (Z)-4-heptenal

($C_7H_{12}O$), 6-methyl-5-hepten-2-one ($C_8H_{14}O$), hexanal ($C_6H_{12}O$), isophorone ($C_9H_{14}O$), and β-ionone ($C_{13}H_{20}O$).

The volatile component (E,E)-2,4-heptadienal is described in literature as a fatty, nutty odor, and 6-methyl-5-hepten-2-one as a sweet, fruity aroma (Qin, Pang, Chen, Cheng, Hu, & Wu, 2013). Additionally, 1-penten-3-ol, hexanal and (E)-2-hexenal all have a green note aroma, and β-cyclocitral and β-ionone were reported as minty and woody odors, respectively (Wang, Wang, Li, Ye, and Kubota, 2010; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013). Isophorone is characterized by a peppermint-like odor (Kataoka, Terada, Inoue, & Mitani, 2007). A creamy odor was associated to (Z)-4-heptenal in a Cheddar cheese study (Zehentbauer & Reineccius, 2002). Lastly, (E)-2-pentenal is described as a fruity, strawberry-like odor (Jordán, Margaría, Shaw, & Goodner, 2002).

The peak areas of the volatile components were compared to analyze the effect of adding salt (NaCl) to vine tea infusions samples. According to Yang, Baldermann, and Watanabe (2013), addition of salt may affect extraction of volatile compounds from tea samples since it can reduce their water solubility (and increase gas-liquid partition), especially for the polar compounds. Paired t-test analysis showed a significant increase in volatile component peak areas ($P<0.05$) when salt was added to vine tea infusions samples (Figure 9). This result suggests addition of NaCl as a good method to optimize the extraction of volatile components in vine tea infusions samples.

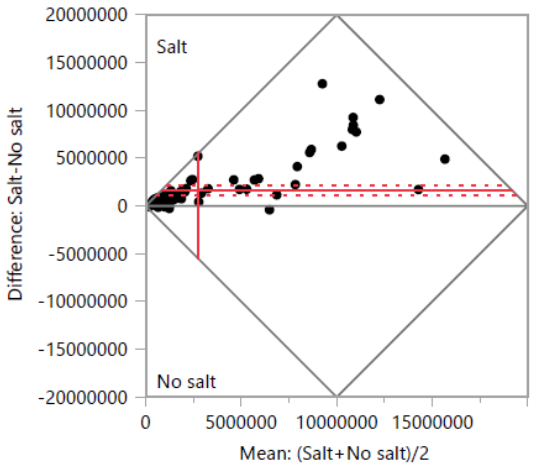


Figure 9. Effect of salt addition on volatile extraction

## 4.4 Vine tea volatile components

Although most studies studying volatile components in teas and herbal teas use distilled (DI) water for extraction, consumers generally use tap water to prepare their infusions. In our study, 56 volatile components present in vine tea infusions brewed with both DI and tap water were identified using SPME with GC-MS and had their Kovats or Linear Retention Index (LIR) confirmed in literature (Table 3). Sensory attributes were not measured in this study, so odor description of the volatile components are provided from available literature.

Table 3. Volatile components in vine tea infusions.

| Number | Component | LRI (Calculated) | LRI (Literature) | Water | | Vine tea | | | Odor description | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DI | TAP | A | B | C | | |
| 1 | Acetone | 829 | 814[7] | x | | x | x | x | - | - |
| 2 | 3-Methylbutanal | 902 | 912[1], 990[3], 917[5], 917[7] | x | x | x | x | x | Fruity, almond, toasted, malty, green, herbal. | 1, 3 |
| 3 | 2-Ethylfuran | 932 | 952[5], 945[7] | x | x | x | x | | - | - |
| 4 | Pentanal | 969 | 935[1,2], 991[3], 977[7] | x | x | x | x | x | Pungent, almond, herbal, green, malty, rubbery, woody, vanilla, nutty. | 1, 2, 3 |
| 5 | Trichloromethane | 1004 | 1018[1] | | x | x | | x | - | - |
| 6 | Hexanal | 1068 | 1067[1], 1084[2], 1097[3], 1081[4], 1089[5], 1064[6], 1080[7] | x | x | x | x | x | Green, fruity, acorn, tallowy, fishy, grassy, herbal, leafy, fatty, apple-like, fresh. | 1, 2, 3, 4, 8 |
| 7 | (E)-2-Pentenal | 1114 | 1117[1], 1104[6], 1135[7] | x | x | | x | x | Pungent, apple, fruity, strawberry, oily, soapy. | 1, 8 |
| 8 | 4-Methyl-3-penten-2-one | 1118 | 1152[1], 1139[2], 1110[6] | x | x | x | | | Sweet, chemical, minty. | 1, 2 |
| 9 | 1-Penten-3-ol | 1137 | 1177[3], 1162[4],1139[6], 1176[7] | x | x | x | x | x | Green, vegetable, milky, butter. | 3, 4, 8 |
| 10 | 5-Methyl-2-heptanone | 1170 | 1252[1] | x | x | x | | | - | - |
| 11 | (Z)-2-Pentenal* | 1184 | 1142[7] | x | x | | x | | - | |
| 12 | (E)-2-Hexenal | 1206 | 1192[2], 1224[3], 1229[4], 1221[5], 1192[6], 1225[7] | x | x | x | x | x | Green, leafy. | 2, 3 |
| 13 | (Z)-4-Heptenal | 1223 | 1253[1], 1218[6] | x | x | x | x | x | Boiled potato, creamy, sweet, biscuit. | 1, 8 |
| 14 | 3-Octanone | 1239 | 1251[7] | x | | x | | | - | - |
| 15 | Octanal | 1269 | 1300[1], 1302[1], 1307[1], 1280[2], 1278[6], 1286[7] | x | x | x | x | x | Lemon, stewed, boiled meat, rancid, soapy, citrus, green, flower, fruity, orange, fatty. | 1,2 |
| 16 | 1-Octen-3-one | 1277 | 1305[1], 1317[1], 1323[1], 1299[7] | x | x | x | x | | Mushroom, metallic, dirty, dust. | 1 |
| 17 | (Z)-2-Heptenal | 1299 | 1319[1], 1320[1] | x | | | x | | - | |
| 18 | 2,2,6-Trimethylcyclohexanone | 1301 | 1320[1] | | x | x | x | | Pungent | 1 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | 6-Methyl-5-hepten-2-one | 1309 | 1319[1], 1347[3], 1337[5], 1320[6], 1340[7] | x | x | x | x | x | Mushroom, earthy, vinyl, rubbery, woody, blackcurrant, boiled fruit, sweet, fruity. | 1, 3 |
| 20 | Nonanal | 1363 | 1402[1], 1408[1], 1415[1], 1385[2], 1397[3], 1368[6], 1396[7] | x | x | x | x | x | Gravy, green, tallowy, fruity, gas, chlorine, floral, waxy, sweet, melon, soapy, fatty, lavender, citrus fruit, oily. | 1, 2, 3 |
| 21 | (E,E)-2,4-Hexadienal | 1368 | 1337[1] | x | x | | x | | Green, vegetable. | 1, 8 |
| 22 | (E)-2-Octenal | 1396 | 1377[1], 1442[1], 1432[7] | | x | | x | x | Fatty, nutty, sweet, sour, waxy, green, burnt, mushroom. | 1, 8 |
| 23 | Acetic acid | 1398 | 1434[1], 1449[1], 1452[1], 1477[1], 1450[2], 1455[5] | x | x | x | x | x | Sour, vinegar, pungent. | 1,2 |
| 24 | α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol (Linalool oxide) | 1405 | 1423[2] | x | x | | x | x | Flower, woody. | 2 |
| 25 | Furfural | 1413 | 1458[1],1455[2], 1466[5] | x | x | | | x | Woody, almond, sweet, fruity, flowery, bread. | 1,2 |
| 26 | (E,Z)-2,4-Heptadienal* | 1424 | 1476[3], 1461[4], 1430[6] | x | x | x | x | x | Fatty, nutty. | 3 |
| 27 | (E,E)-2,4-Heptadienal | 1457 | 1502[3,] 1489[4], 1494[5], 1456[6], 1497[7] | x | x | x | x | x | Fatty, oily. | 3 |
| 28 | Decanal | 1464 | 1447[1], 1510[1], 1517[1], 1538[1], 1484[2], 1502[7] | x | | x | x | x | Stewed, burnt, green, waxy, Floral, lemon, fatty, herbal, soapy, orange peel, tallowy. | 1, 2 |
| 29 | Benzaldehyde | 1482 | 1500[1], 1522[1], 1525[1], 1495[2], 1536[3], 1515[4], 1526[5], 1482[6], 1528[7] | x | x | | | x | Burnt sugar, almond, woody, fragrant, sweet, almond. | 1, 2, 3,4 |
| 30 | (E,Z)-3,5-Octadien-2-one* | 1484 | 1484[6] | x | x | x | x | x | Synthetic, plastic. | 8 |
| 31 | Linalool | 1500 | 1537[2], 1549[3], 1540[4], 1548[5], 1522[6], 1554[7] | x | x | x | x | x | Flower, lavender, floral, sweet. | 2, 3,4 |
| 32 | 2-Methylpropanoic acid | 1515 | 1584[1], 1588[1], 1570[5] | x | | x | | | Cheesy, phenolic, fatty, sweaty. | 1 |
| 33 | (E,E)-3,5-Octadien-2-one | 1533 | 1521[1,5], 1576[4], 1539[6] | x | x | x | x | x | Fresh, sweet, woody, mushroom. | 1, 8 |
| 34 | (E,Z)-2,6-Nonadienal | 1548 | 1597[1], 1605[1] | x | | x | x | x | Cucumber, melon. | 1, 8 |
| 35 | 6-Methyl-3,5-heptadien-2-one | 1559 | 1598[5] | | x | x | | x | - | - |
| 36 | 2,6-Dimethylcyclohexanol | 1564 | 1598[4] | x | x | x | x | x | - | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 37 | Undecanal | 1569 | 1624[1] | x | | x | x | x | Fruity, green, waxy, oily. | [1] |
| 38 | β-Cyclocitral | 1584 | 1598[2], 1620[3], 1611[4], 1593[6] | x | x | x | x | x | Minty, mild green, fruity. | [2, 3, 4] |
| 39 | Safranal | 1607 | 1648[2], 1633[5], 1596[6] | x | x | x | | x | Herb, sweet | [2] |
| 40 | 3-Methylbutanoic acid (isovaleric acid) | 1611 | 1660[1], 1665[1], 1686[1], 1691[1], 1665[2], 1669[5], 1631[6] | x | | x | | x | Sweaty, cheesy, rancid, acid. | [1, 2] |
| 41 | 2-Methylbutanoic acid | 1616 | 1667[1], 1670[5] | | x | x | | x | Overripe fruit, sweaty, cashew, sweet. | [1] |
| 42 | 2,6,6-Trimethyl-2-cyclohexene-1,4-dione | 1659 | 1655[6] | x | x | x | | | - | - |
| 43 | α-Terpineol | 1660 | 1688[2], 1692[3], 1695[4], 1662[6] | x | x | | | x | Oily, anise, minty, floral, lilac-like. | [2,3] |
| 44 | Dodecanal | 1678 | 1700[1], 1718[1], 1722[1], 1728[1] | x | | x | x | x | Oily, herbal, fatty, citrus, waxy. | [1] |
| 45 | α-Citral | 1701 | 1686[1], 1708[1], 1744[1], 1755[1], 1693[6] | x | x | | x | | Floral, lemon, minty, pungent, fruity. | [1] |
| 46 | 2,4-Dimethylbenzaldehyde | 1702 | 1710[1] | x | x | x | | | Cherry, almond, spicy, vanilla. | [1] |
| 47 | (E,E)-2,4-Decadienal | 1731 | 1702[1], 1778[1], 1820[1], 1832[1], 1777[6] | x | | | x | | Fatty, waxy, deep-fried, pungent, green, citrus | [1] |
| 48 | Methyl salicylate | 1743 | 1745[2], 1779[3], 1766[4], 1740[5], 1727[6] | | x | x | x | x | Wine, berry, warm, sweet, wintergreen, peppermint, minty. | [1, 2,3,4] |
| 49 | Tridecanal | 1789 | 1772[1], 1792[1], 1824[1], 1824[2] | x | | x | x | x | Fresh, soapy, citrus, waxy, grapefruit peel, flower, sweet, must. | [1, 2] |
| 50 | Hexanoic acid (Caproic acid) | 1796 | 1847[1], 1863[1], 1872[1], 1829[2], 1845[4], 1777[5], 1807[6] | x | x | x | x | x | Sweaty, pungent, cheesy, goat-like, rancid. | [1, 2] |
| 51 | α-Ionone | 1827 | 1809[2], 1842[4], 1818[6] | x | x | x | x | x | Woody, violet-like, floral. | [2, 4] |
| 52 | Heptanoic acid | 1915 | 1990[1,] 1955[4], 1887[6] | x | | | | | Fatty, sour, sweaty, rancid. | [1] |
| 53 | β-Ionone | 1925 | 1912[2], 1909[4],1889[6] | x | x | x | x | x | Seaweed, violet, flower, raspberry, woody. | [2, 4] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 54 | 5,6-Epoxy-β-ionone | 1983 | 1984[4], 1954[6] | x | x | x | x | x | - | - |
| 55 | Octanoic acid | 2028 | 2083[1], 2098[1], 2083[2], 2065[4], 2013[6] | x | | x | | x | Fatty, cheesy, fresh, moss, sweaty | [1, 2] |
| 56 | Nonanoic acid | 2142 | 2202[1], 2202[2], 2158[4], 2096[6] | x | | x | x | x | Green, fat, musty, sweaty, sour | [1, 2] |

[1] Pherobase database (http://www.pherobase.com/). [2] Flavornet database (http://www.flavornet.org/flavornet.html). [3] Qin, Pang, Chen, Cheng, Hu, & Wu (2013). [4] Wang, Wang, Li, Ye, & Kubota (2010). [5] Kraujalytė, Pelvan, & Alasalvar (2016). [6] Kawakami, Ganguly, Banerjee, & Kobayashi (1995). [7]Bianchi, Careri, Mangia, & Musci (2007). [8] Venkateshwarlu, Let, Meyer, & Jacobsen (2004).

GC–MS is the most efficient method to separate, identify, and quantify volatile compounds (Yang, Baldermann, & Watanabe, 2013). The total number of volatile components identified from vine tea samples brewed with DI and tap water were fifty and forty-two, respectively. Of these, thirty-six compounds were found in both.

More than a few volatile components identified in the vine tea samples are previously recognized components of other teas and herbal teas flavors. For instance, hexanal, a fatty acid-derived volatile (green, grassy, metallic odor), linalool, a volatile terpene (floral, citrus-like odor), and β-ionone, a carotenoid-derived volatile (woody, violet-like odor), are some characteristic flavor compounds in tea infusions (Yang, Baldermann, & Watanabe, 2013).

Hexanal is a key odorant in expresso coffee and has been reported in black, green and oolong teas and herbal teas such as rooibos and cocoa teas (Lasekan & Lasekan, 2012; Maeztu, Sanz, Andueza, Pena, Bello, & Cid, 2001; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Sheibani, Duncan, Kuhn, Dietrich, Newkirk, & O'Keefe, 2015; Wang, Wang, Li, Ye, & Kubota, 2010). The green odor associated to hexanal also describes other aldehydes such as octanal and nonanal, which were also found in oolong tea (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Zehentbauer & Reineccius, 2002). Besides hexanal, (E)-2-octenal (fatty, nutty) and (E,E)-2,4-decadienal (fatty, deep-fried) are other aldehydes identified in vine tea infusions that are resulting from linoleic acid degradation (Cerny, 2010).

The volatile component 3-methylbutanal (malty odor and also known as isovaleraldehyde) was reported as one of the key odorants of expresso coffee, and brews prepared from arabica and robusta coffees, and it is also an odorant of cocoa mass (Cheetham, 2002; Maeztu, Sanz, Andueza, Pena, Bello, & Cid, 2001). Pentanal (woody, vanilla, nutty odor) was reported in mango fruits, black and green teas (Pino, Mesa, Muñoz, Martí, & Marbot, 2005;

Qin, Pang, Chen, Cheng, Hu, & Wu, 2013). Safranal was also present in black tea, as well as other volatile components identified in vine tea infusions, such as hexanoic acid and 2-ethylfuran (Kraujalytė, Pelvan, & Alasalvar, 2016).

Linalool (floral, sweet odor) is another volatile component identified in vine tea infusions which is widespread in plant volatiles. It has been identified as flavor compound of rosemary and peppermint infusions, jasmine, chamomile, cocoa, green and black teas, and it is also an important volatile component in tomato (Buttery, 1993; Lasekan & Lasekan, 2012; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Riachi, Abi-Zaid, Moreira, & Maria, 2012; Tschiggerl & Bucar, 2010, 2012; Wang, Wang, Li, Ye, & Kubota, 2010).

Linalool oxide (green, floral, fruity, sweet odor) was previously identified in oolong tea and 2,6-dimethylcyclohexanol (odor description not found) was also reported in cocoa tea infusion (Sheibani, Duncan, Kuhn, Dietrich, Newkirk, & O'Keefe, 2015; Wang, Wang, Li, Ye, & Kubota; 2010).

Among the ketones identified in vine tea infusions, α-ionone (woody odor) was also present in roasted mate and both β-ionone (floral odor) and 5,6-epoxy-β-ionone (woody) were found in rooibos tea (Lasekan & Lasekan, 2012). All three components were reported in cocoa tea, and both α-ionone and β-ionone were identified in black, green and oolong teas (Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Sheibani, Duncan, Kuhn, Dietrich, Newkirk, & O'Keefe, 2015; Wang, Wang, Li, Ye, & Kubota, 2010). The ketone 1-octen-3-one (mushroom odor) is an important odorant of milk chocolate and was also reported in mild Cheddar cheese (Cheetham, 2002; Zehentbauer & Reineccius, 2002). The isomers (E,Z)-3,5-octadien-2-one (synthetic, plastic odor) and (E,E)-3,5-octadien-2-one (fresh, sweet odor) were both found in oolong tea and

fish oil enriched milk (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Venkateshwarlu, Let, Meyer, & Jacobsen; 2004).

Moreover, acetic acid (sour odor) has been reported in Turkish Tombul hazelnuts, coffee drinks, black tea, peppermint infusions, and is also a key compound in formulation of sweet cream butter aroma (Alasalvar, Shahidi, & Cadwallader, 2003; Cheetham, 2002; Kraujalytė, Pelvan, & Alasalvar, 2016; Kumazawa, 2006; Riachi, Abi-Zaid, Moreira, & Maria, 2012). Acetic and hexanoic acids are also two of the main components of formulations of natural black cherry flavor (Cheetham, 2002).

The following volatile components were identified only in vine tea infusions brewed with DI water: acetone; 3-octanone; (Z)-2-heptenal; (E,Z)-2,6-nonadienal; (E,E)-2,4-decadienal; decanal; undecanal; dodecanal; tridecanal; 2-methylpropanoic acid; 3-methylbutanoic acid; heptanoic acid; octanoic acid; and nonanoic acid. Acetone was also found in Italian sausage "Salame Mantovano" and 3-octanone was present in rosemary infusions (Bianchi, Careri, Mangia, & Musci, 2007; Tschiggerl & Bucar, 2010). The aldehyde (Z)-2-heptenal was reported as a volatile component from leaves, olives, and virgin oil of *Olea europaea* cultivar Olivastra Seggianese (Flamini, Cioni, & Morelli, 2003). In addition, (E,Z)-2,6-nonadienal and (E,E)-2,4-decadienal are important aroma compounds in apricot and cucumber, respectively (Buttery, 1993).

Decanal (waxy, soapy, flowery odor) was found in oolong tea, mild Cheddar cheese, and it was also reported as one of the main contributors to cooked California long-grain rice aroma (Buttery, 1993; Sheibani, Duncan, Kuhn, Dietrich, Newkirk, & O'Keefe, 2015; Zehentbauer & Reineccius, 2002). Undecanal (odor not described), octanal (fragant, citrus odor) and decanal (citrus odor) were reported in fish oil enriched milk (Venkateshwarlu, Let, Meyer, & Jacobsen,

2004). Dodecanal is a component of conventionally prepared and poroplast-extracted hydrocarbon-free orange oils, as well as octanal and decanal (Cheetham, 2002). Also, tridecanal, undecanal, decanal, octanal and 2-methylpropanoic acid were volatile components reported in mango fruits (Pino, Mesa, Muñoz, Martí, & Marbot, 2005).

Heptanoic, octanoic and nonanoic acids have been reported in cocoa tea and oolong tea, and the last two were also found in peppermint infusions (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Riachi, Abi-Zaid, Moreira, & Maria, 2012; Wang, Wang, Li, Ye, and Kubota, 2010). Both nonanoic and isovaleric acids were found in black tea and mango fruits (Kawakami, Ganguly, Banerjee, and Kobayashi, 1995; Pino, Mesa, Muñoz, Martí, & Marbot, 2005). Nonanoic acid (fatty, green odor) was also present in green tea, and 3-methylbutanoic acid (sour, sweaty odor and also known as isovaleric acid) was reported as a potent odorant in coffee drinks and an aroma compound of milk chocolate (Cheetham, 2002; Kumazawa, 2006; Lee, Chambers, Chambers, Adhikari, & Yoon, 2013).

On the other hand, trichloromethane; 2,2,6-trimethylcyclohexanone;(E)-2-octenal; 6-methyl-3,5-heptadiene-2-one; 2-methylbutanoic acid; and methyl salicylate were identified only in vine tea infusions prepared with tap water.

Tricloromethame ($CHCl_3$), also known as chloroform, has a typical odor and its boiling point at 101.3 kPa is 61.3 °C. It can be found in drinking-water and may be from direct contamination of the water source or may be formed during water chlorination process (WHO, 2004). This halogen compound was also identified worldwide in several foodstuffs, including olive oil, coffee, soft drinks, pork, milk, and Fontina Valle d'Aosta cheese, and its taste is described as burning, sweet. (Bianchi, Careri, Mangia, & Musci, 2007; WHO, 2004).

The ketone 6-methyl-3,5-heptadiene-2-one (odor description not found) is also present in black tea, and 2,2,6-trimethylcyclohexanone (pungent odor) was reported in apricot (Gómez, Ledbetter, & Hartsell, 1993; Kraujalytė, Pelvan, & Alasalvar, 2016). Additionally, (E)-2-octenal (fatty, waxy odor) is an odorant of cocoa mass and 2-methylbutanoic acid (sweaty odor) is an aroma compound of milk chocolate and apricot (Cheetham, 2002). Methyl salicylate (minty odor) is found in black tea and cocoa tea infusions, and it is one of the key aroma compounds in tomato (Buttery, 1993; Kraujalytė, Pelvan, & Alasalvar, 2016; Wang, Wang, Li, Ye, & Kubota, 2010).

From the results described above, the type of water clearly affects the volatile chemical composition of vine tea infusions and further sensory studies are suggested to evaluate if this difference is noticeable to consumers and affects vine tea acceptability. Kovalenko and Vietrov (2015) reported water hardness affects taste and aroma of black and green teas drinks. Residual chlorine also had a significant negative effect on smell and taste of black and green teas drinks (Kovalenko & Vietrov, 2015).

Moreover, seven aldehydes (hexanal, (E)-2-hexenal, (Z)-4-heptenal, nonanal, (E,Z)-2,4-heptadienal, (E,E)-2,4-heptadienal, and β-cyclocitral), two ketones (6-methyl-5-hepten-2-one, and β-ionone), and one alcohol (1-penten-3-ol) were identified in more than 90% of all vine tea infusions. Both ketones, the alcohol and five of the aldehydes listed above (hexanal, (E)-2-hexenal, nonanal, (E,E)-2,4-heptadienal, and β-cyclocitral) were also reported in black and oolong teas, and the other two aldehydes (Z)-4-heptenal and (E,Z)-2,4-heptadienal were reported in oolong and black teas, respectively (Kawakami, Ganguly, Banerjee, and Kobayashi, 1995).

Other studies have reported 1-penten-3-ol (leafy sweet odor) in green tea, green mate and cocoa teas (Lasekan & Lasekan, 2012; Lee, Chambers, Chambers, Adhikari, & Yoon, 2013;

Wang, Wang, Li, Ye, & Kubota, 2010). This alcohol was also reported as an important volatile component in tomato, as well as 6-methyl-5-hepten-2-one, β-ionone, β-cyclocitral, (E)-2-hexenal and hexanal (Buttery, 1993).

The aldehyde (Z)-4-heptenal (metallic, hay-like odor) was also found in green tea, and (E)-2-hexenal (fruity, strawberry, cherry odor) was present in oolong tea, cocoa tea, kiwi essence and kiwi fruit puree (Jordán, Margaría, Shaw, & Goodner, 2002; Kumazawa, 2006; Sheibani, Duncan, Kuhn, Dietrich, Newkirk, & O'Keefe, 2015; Wang, Wang, Li, Ye, & Kubota, 2010). In addition, hexanal (grassy odor), (E,E)-2,4-heptadienal (fatty, oily odor) and nonanal (fatty, oily odor) were also identified in green and black teas, and in natural and roasted Turkish Tombul hazelnuts (*Corylus avellana* L.) (Alasalvar, Shahidi, & Cadwallader, 2003; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013).

The isomers (E,Z)-2,4-heptadienal (fatty, nutty odor) and (E,E)-2,4-heptadienal (fatty, oily odor) were both present in fish oil enriched milk, black, green, and cocoa teas (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Venkateshwarlu, Let, Meyer, & Jacobsen, 2004; Wang, Wang, Li, Ye, & Kubota, 2010). Also, (E,E)-2,4-heptadienal (orange oil, oily odor) was reported in oolong tea, kiwi essence and kiwi fruit puree (Jordán, Margaría, Shaw, & Goodner, 2002; Kawakami, Ganguly, Banerjee, & Kobayashi, 1995).

Overall, the vine tea sample (brands A, B and C), water quality (distilled and tap waters) and the interaction factor had significant effects on the peak area of the twelve volatile components noted above ($P<0.05$). However, these factors had different impacts on the peak area of each volatile component. β-cyclocitral (mild green, minty, fruity odor) was the only volatile component whose peak area was not significantly affected by water type ($P>0.05$) (Qin, Pang,

Chen, Cheng, Hu, & Wu, 2013). Additionally, vine tea sample and interaction factor did not have a significant on the peak area of β-ionone ($P>0.05$), which odor is reported as a woody, floral (rose, violet) (Qin, Pang, Chen, Cheng, Hu, & Wu, 2013). Results of the effect tests and mean comparisons using Tukey's HSD test are shown in Appendices E and F, respectively.

Some components were uniquely identified in infusions brewed from brand A, which was the aged tea: 4-methyl-3-penten-2-one, 5-methyl-2-heptanone, 2-methylpropanoic acid, 2,6,6-trimethyl-2-cyclohexene-1,4-dione, and 2,4-dimethylbenzaldehyde.

The ketone 4-methyl-3-penten-2-one was also found in oolong and black teas, and 5-methyl-2-heptanone was present in natural and roasted Turkish Tombul hazelnuts (*Corylus avellana* L.) (Alasalvar, Shahidi, & Cadwallader, 2003; Kawakami, Ganguly, Banerjee, & Kobayashi, 1995). The volatile components 2-methylpropanoic acid and 2,6,6-trimethyl-2-cyclohexene-1,4-dione were reported in black tea in oolong tea, resapectively (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Kraujalytė, Pelvan, & Alasalvar, 2016). In addition, 2,4-dimethylbenzaldehyde was found in murici (*Byrsonima crassifolia* L. Rich), a small round fruit found in the Northern and Northeastern regions of Brazil (Alves & Franco, 2003).

Four aldehydes were identified only from vine tea infusions prepared with brand B samples: (E)-2-pentenal, (E,E)-2,4-hexadienal, (Z)-2-heptenal, and (E,E)-2,4-decadienal. The volatile compound (Z)-2-heptenal was found in safflower (*Carthamus tinctorius* L.) flowerhead buds and (E)-2-pentenal was found in oolong tea, kiwi essence and kiwi fruit puree (Binder, Benson, & Flath, 1990; Jordán, Margaría, Shaw, & Goodner, 2002; Kawakami, Ganguly, Banerjee, & Kobayashi, 1995). Also, (E,E)-2,4-hexadienal was reported in fish oil enriched milk and (E,E)-2,4-decadienal is one of the major components of cooked California long-grain rice

aroma and was also reported in oolong tea (Buttery, 1993; Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Venkateshwarlu, Let, Meyer, & Jacobsen, 2004).

In addition, furfural, benzaldehyde, and α-terpineol were solely identified in infusions from brand C samples, which were ground dried leaves and stems of vine tea originally packed in tea bags. Both furfural (nutty, chocolate, earthy odor) and benzaldehyde (nutty odor) were present in panned oolong tea and have been reported as important volatile components in tomato (Sheibani, Duncan, Kuhn, Dietrich, Newkirk, & O'Keefe, 2015; Buttery, 1993). Benzaldehyde (fragrant, sweet, almond odor) is a typical component for formulation of natural black cherry flavor and was also reported in black, oolong, green and cocoa teas (Cheetham, 2002; Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Lee, Chambers, Chambers, Adhikari, & Yoon, 2013; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Wang, Wang, Li, Ye, & Kubota, 2010). The alcohol α-terpineol (sweet ether-like odor) has been identified as flavor compound in peppermint and rosemary infusions, green mate, jasmine, chamomile, green, oolong and black teas (Kawakami, Ganguly, Banerjee, & Kobayashi, 1995; Lasekan & Lasekan, 2012; Qin, Pang, Chen, Cheng, Hu, & Wu, 2013; Riachi, Abi-Zaid, Moreira, & Maria, 2012; Tschiggerl & Bucar, 2010, 2012).

Several volatile components identified in the chromatograms of vine tea infusions were considered contaminants because they were also identified in the chromatograms of empty vials (blanks). Most of these volatile components were silicone compounds that possible came from the GC septum. We studied the source and used septa of different polymeric materials (polytetrafluoroethylene (PTFE)/silicone and PTFE/chlorobutyl) with the screw caps. The contaminants were found in all samples and were therefore, most likely arising from the silicone septa used in the injection port and the relatively wide needles used for SPME. Some examples

of the contaminants identified included: hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, decamethylcyclopentasiloxane, dodecamethylcyclohexasiloxane, tetradecamethylcycloheptasiloxane, decamethyltetrasiloxane, dodecamethylpentasiloxane, tetradecamethylhexasiloxane, hexadecamethylheptasiloxane, and dibutyl phthalate.

Other thrity-nine volatile components that were identified only by mass spectra in vine tea infusions are reported in Table 4:

Table 4. Additional volatile components identified by mass spectrometry in vine tea infusions.

| Number | Component | LRI (Calculated) | Water | | Vine tea | | |
|---|---|---|---|---|---|---|---|
| | | | DI | TAP | A | B | C |
| 1 | 3,4-Dimethyl-2-pentene | 1117 | x | x | x | | |
| 2 | 5-Methylhexanal | 1171 | x | x | | x | x |
| 3 | 5-Methyl-3-heptanone | 1238 | | x | | x | |
| 4 | Pentanoyl chloride | 1247 | x | | | x | |
| 5 | (E)-3-Decen-1-yne | 1268 | x | | | x | |
| 6 | 2-Methyl-1-hepten-6-one | 1292 | x | x | x | x | |
| 7 | Cyclodecane | 1301 | x | | x | | |
| 8 | Isophorone | 1374 | x | x | x | x | x |
| 9 | 3-Ethyl-2-methyl-1,3-hexadiene | 1383 | | x | | x | |
| 10 | 2,6,6-Trimethyl-2-cyclohexene-1-carboxaldehyde | 1405 | x | x | x | | |
| 11 | 3,4,4-Trimethyl-2-cyclohexen-1-one | 1416 | x | x | x | x | |
| 12 | 3,7-Dimethyl-1-octanol | 1486 | x | | | x | |
| 13 | 10-Methylundec-3-en-4-olide | 1504 | x | x | x | x | |
| 14 | 1-(2-methyl-1-cyclopenten-1-yl)ethanone | 1557 | x | x | x | x | x |
| 15 | 2-Allylfuran* | 1573 | x | | | x | |
| 16 | 4-Methyl-2,4,6-cycloheptatriene-1-one * | 1590 | x | | | x | |
| 17 | Tetramethylbutanedinitrile | 1592 | x | x | | | x |
| 18 | 2-Methyl-7-oxabicyclo[2.2.1]heptane* | 1650 | x | | | x | |
| 19 | 1,1-Dimethyl-2-(1-methyl-2-propenyl)cyclopropane | 1652 | | x | | x | |
| 20 | (E)-4-Oxohex-2-enal | 1725 | x | x | x | x | x |
| 21 | 4-Propylbenzaldehyde | 1799 | x | | x | x | x |
| 22 | (Z)-6,10-Dimethyl-5,9-undecadien-2-one | 1825 | x | x | x | x | x |
| 23 | 6,10-Dimethyl-5,9-undecadien-2-one | 1826 | x | x | x | x | x |
| 24 | Butyl carbamate | 1832 | x | | x | x | x |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | 2-Methyl-1-(1,1-dimethylethyl)-2-methylpropanoic acid, 1,3-propanediyl ester | 1857 | x | x | x | x | x |
| 26 | 1-Butenylidene-cyclohexane | 1865 | x | x | x | x | x |
| 27 | (E,Z)-4,5-diethyl-3,5-octadiene | 1893 | | x | x | x | |
| 28 | 1-(1H-pyrrol-2-yl)ethanone | 1937 | x | | x | | |
| 29 | Dehydro-β-ionone | 1988 | | x | x | | x |
| 30 | Diisobutyl adipate | 2121 | x | | x | x | x |
| 31 | 8a-Methylhexahydro-1,8(2H,5H)-naphthalenedione | 2151 | x | x | x | x | x |
| 32 | 1,3-Cyclohexanedione, 2-(2-propenyl)- | 2152 | | x | x | x | x |
| 33 | Dicyclohexylmethanone | 2190 | x | x | x | | |
| 34 | 2-tert-Butyl-4-methylphenol | 2215 | | x | | x | x |
| 35 | 2,2',5,5'-Tetramethylbiphenyl | 2260 | | x | | x | x |
| 36 | 3,5-bis(1,1-Dimethylethyl)phenol | 2272 | | x | x | x | x |
| 37 | 2-Ethylhexyl salicylate | 2301 | | x | x | x | x |
| 38 | 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone | 2377 | x | x | x | x | x |
| 39 | (4-methylphenyl)phenyl methanone | >2377 | | x | | x | x |

*First match was rejected after compared to available LRI (literature).

Two geranylacetone ((E)-6,10-dimethyl-5,9- undecadien-2-one) stereoisomers, (Z)-6,10-dimethyl-5,9-undecadien-2-one and 6,10-dimethyl-5,9-undecadien-2-one, were identified in vine tea infusions by mass spectrometry and their calculated LRI were 1825 and 1826, respectively. These LRI values were not confirmed in literature, as shown in Table 4. However, Kawakami, Ganguly, Banerjee, and Kobayashi (1995) analyzed different extracts of oolong teas and reported the Kovats index (Carbowax 20 column) of geranylacetone was equal to 1820, which is very close to the results found for geranylacetone stereoisomers identified in vine tea infusions.

Dehydro-β-ionone (woody-flora odor) was reported in Thai flowers (*Michelia champacca*) and isophorone was present among the volatile components of mango fruits (Lasekan & Lasekan, 2012; Pino, Mesa, Muñoz, Martí, & Marbot, 2005). However, LRI on Carbowax 20 or equivalent column were not found for either component.

Also, the volatile component identified as 5-methyl-3-heptanone in Table 4 had retention time and LRI very close to the volatile component identified as 3-octanone in Table 3. The mass spectra obtained from vine tea infusions were very similar to both 5-methyl-3-heptanone (first match) and 3-octanone (second match) mass spectra. This result suggests the unknown component reported as 5-methyl-3-heptanone could also be 3-octanone. One alternative to confirm this hypothesis would be comparing the mass spectra and LRI of the sample to certified standards and separate using a column of different polarity.

In conclusion, mass spectrometry results alone do not provide a complete identification of volatile components. Thus, further investigation is needed to confirm the additional thirty-nine components reported in Table 4 are truly present in vine tea infusions.

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# CHAPTER 5

# CONCLUSIONS

Headspace solid-phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) were used to identify volatile components of vine tea infusions, which typically had acidic pH values and dark, reddish-yellow color. As expected, type of water (distilled and Blacksburg tap water) and vine tea sample (brands A, B and C) both affected the overall volatile chemical composition of vine tea infusions and different volatile profiles were observed among the infusions. The impact of water quality is relevant to the food industry and may be considered for the development of new healthy products, such as ready-to-drink beverages. Thus, further studies are suggested to understand which water composition would result vine tea infusions with the highest nutrition values and optimum sensorial attributes (flavor, taste and color).

Additionally, vine tea is still mostly produced from wild plants in China. This factor plus different processing conditions may be the main causes of such diverse volatile profiles observed in commercial vine tea samples. Only twelve volatile components were found in all combinations of vine tea samples and water type. However, before assuming they would be the main volatile components in vine tea flavor profile, additional qualitative and sensorial studies should be performed, especially gas chromatography-olfactometry (GC-O).

This study was performed using commercial samples that have vine tea as their only ingredient listed. Even though problems related to quality control of Chinese herbal teas have been reported in literature, it was assumed the analyzed samples were truly 100% vine tea. Also, information about harvest and processing conditions of these samples are unknown. Both production and processing conditions may affect the volatile profile of products from plant

sources, such as herbal teas. For this reason, further studies are suggested to be performed using samples from controlled harvest and processing.

Several volatile components identified in vine tea infusions are also known components of the aroma profile of other teas and herbal teas largely consumed worldwide, such as green, black, oolong, mate, chamomile and rooibos teas. The presence of those components may contribute positively to overall acceptability of vine tea drinks in other places where it is still not very known as the United States. However, consumer studies are highly recommended to a better understanding of vine tea acceptability in those places.

Furthermore, some volatile components present in vine tea infusions were identified only by mass spectrometry, because published Kovats index were not found for the Carbowax 20M or its equivalent columns. This result alone is considered weak and additional confirmation of these volatile components is needed. In this study only a polar GC column (ZB-Wax plus) was used to analyze vine tea infusions. So, one alternative would be to perform a similar study using a nonpolar GC column, for example a DB-5 column, with further confirmation of calculated Kovats index. Another option would be running certified standards and comparing their results (retention time and mass spectra) with the results of the unknown volatiles from vine tea infusions.

In conclusion, there are still many questions to be answered about vine tea quality attributes, such as color, aroma and flavor. This study provides an initial picture of the volatile profile of vine tea infusions and may direct further investigations of this antioxidant-rich herbal tea.

# APPENDICES

Appendix A – Certificate of composition of *n*-Paraffin Mix C5, C6, C7, C8.

# Certificate of Composition

DESCRIPTION: n-Paraffin Mix C5,C6,C7,C8

CATALOG NO.: 47100 MFG. DATE: Aug 2013
LOT NO.: LC02377 EXP. DATE: Aug 2016

| ANALYTE | CAS NO. | PURITY (1) | WEIGHT% (2) | SUPELCO LOT NO. |
|---|---|---|---|---|
| PENTANE | 109-66-0 | 99.9 | 21.28 | LB06672 |
| HEXANE | 110-54-3 | 99.5 | 23.43 | LC01486 |
| N-HEPTANE | 142-82-5 | 99.9 | 25.53 | LC01676 |
| N-OCTANE | 111-65-9 | 99.4 | 29.77 | LB63797 |

(1) Determined by GC-FID unless otherwise noted.
(2) Weight percent of analyte, calculated by using analyte weights. The total may not equal 100% due to rounding. Weight concentrations may not remain stable after opening, even if resealed. NIST-Traceable weights are used to verify balance calibration with the preparation of each lot.

Duane Funk

Duane Funk
Quality Manager

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595 North Harrison Road
Bellefonte, PA 16823-0048 USA
Phone (814) 359-3441

Appendix B – Certificate of composition of *n*-Paraffin Mix C10, C12, C14, C16.

# Certificate of Composition

DESCRIPTION: n-Paraffin Mix C10,C12,C14,C16

CATALOG NO.: 47102 MFG. DATE: Apr 2014
LOT NO.: LC-07376 EXP. DATE: Apr 2017

| ANALYTE | CAS NO. | PURITY (1) | WEIGHT% (2) | SUPELCO LOT NO. |
|---|---|---|---|---|
| N-DECANE | 124-18-5 | 99.7 | 16.63 | LB91079 |
| N-DODECANE | 112-40-3 | 99.9 | 22.24 | LB97934 |
| N-TETRADECANE | 629-59-4 | 99.9 | 27.75 | LC04974 |
| N-HEXADECANE | 544-76-3 | 99.5 | 33.37 | LB87053 |

(1) Determined by GC-FID unless otherwise noted.
(2) Weight percent of analyte, calculated by using analyte weights. The total may not equal 100% due to rounding. Weight concentrations may not remain stable after opening, even if resealed. NIST-Traceable weights are used to verify balance calibration with the preparation of each lot.

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Appendix C – Certificate of composition of *n*-Paraffin Mix C18, C20, C22, C24.

# Certificate of Composition

DESCRIPTION: n-Paraffin Mix C18,C20,C22,C24

CATALOG NO.: 47108 MFG. DATE: Apr 2014
LOT NO.: LC06136 EXP. DATE: Apr 2017

| ANALYTE | CAS NO. | PURITY (1) | WEIGHT% (2) | SUPELCO LOT NO. |
|---|---|---|---|---|
| N-OCTADECANE | 593-45-3 | 98.0 | 2.00 | LB89629 |
| N-EICOSANE | 112-95-8 | 97.9 | 2.04 | LB97320 |
| N-DOCOSANE | 629-97-0 | 99.9 | 2.00 | LB31603 |
| N-TETRACOSANE | 646-31-1 | 99.9 | 2.00 | LB92913 |
| N-OCTANE | 111-65-9 | 99.4 | 91.95 | LB63797 |

(1) Determined by GC-FID unless otherwise noted.
(2) Weight percent of analyte, calculated by using analyte weights. The total may not equal 100% due to rounding. Weight concentrations may not remain stable after opening, even if resealed. NIST-Traceable weights are used to verify balance calibration with the preparation of each lot.

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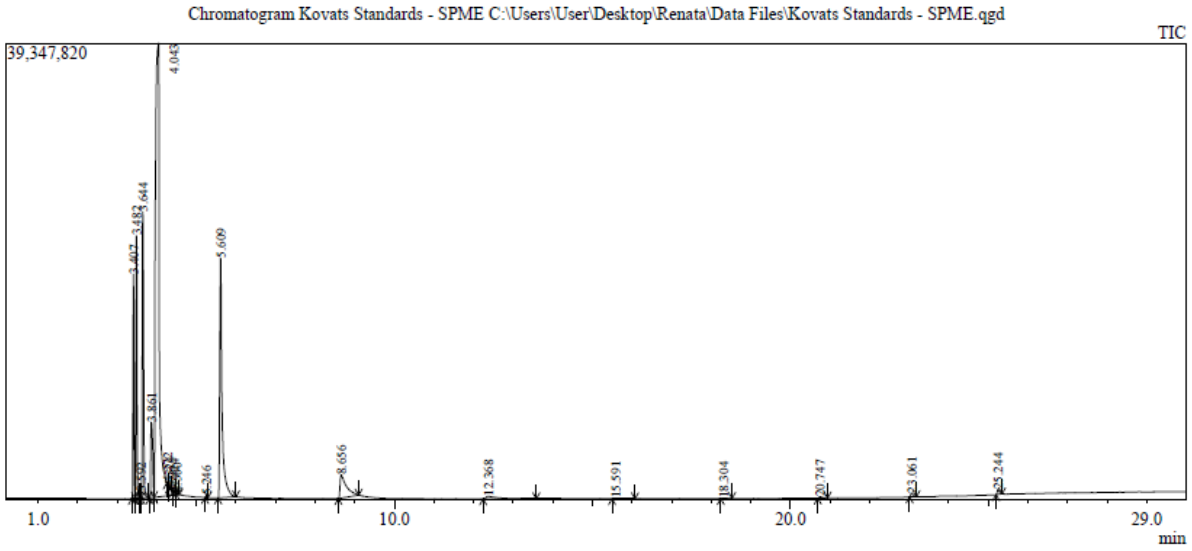
Appendix D – Chromatogram of *n*-alkanes standards (C5-C8, C10, C12, C14, C16, C18, C20, C22 and C24) on a carbowax capillary column (ZB-Wax plus).




Peak Report TIC

| Peak# | R.Time | I.Time | F.Time | Area | Area% | Height | Height% | A/H | Mark | Name |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3.407 | 3.370 | 3.450 | 32868067 | 5.97 | 19340102 | 14.03 | 1.70 | | Pentane |
| 2 | 3.482 | 3.450 | 3.555 | 42421386 | 7.70 | 22664596 | 16.45 | 1.87 | V | n-Hexane |
| 3 | 3.592 | 3.555 | 3.610 | 1035178 | 0.19 | 538804 | 0.39 | 1.92 | V | Cyclopentane, methyl- |
| 4 | 3.644 | 3.610 | 3.795 | 55337006 | 10.04 | 24694716 | 17.92 | 2.24 | V | Heptane |
| 5 | 3.861 | 3.815 | 3.925 | 22420821 | 4.07 | 6513072 | 4.73 | 3.44 | | Heptane, 3-methyl- |
| 6 | 4.043 | 3.925 | 4.565 | 278636106 | 50.58 | 39159112 | 28.41 | 7.12 | SV | Octane |
| 7 | 4.272 | 4.250 | 4.300 | 342361 | 0.06 | 232804 | 0.17 | 1.47 | T | 2-Octene, (E)- |
| 8 | 4.324 | 4.305 | 4.370 | 226949 | 0.04 | 140903 | 0.10 | 1.61 | T | 2-Heptene, 3-methyl- |
| 9 | 4.424 | 4.395 | 4.460 | 548731 | 0.10 | 351160 | 0.25 | 1.56 | T | Cyclopentane, propyl- |
| 10 | 4.500 | 4.470 | 4.535 | 147384 | 0.03 | 91193 | 0.07 | 1.62 | T | Cyclohexane, 1,2-dimethyl-, cis- |
| 11 | 5.246 | 5.215 | 5.290 | 284477 | 0.05 | 183930 | 0.13 | 1.55 | | Nonane, 3-methyl- |
| 12 | 5.609 | 5.545 | 6.000 | 85097442 | 15.45 | 20686697 | 15.01 | 4.11 | | Decane |
| 13 | 8.656 | 8.585 | 9.095 | 22486564 | 4.08 | 2001902 | 1.45 | 11.23 | | Dodecane |
| 14 | 12.368 | 12.240 | 13.585 | 4327961 | 0.79 | 214416 | 0.16 | 20.18 | MI | Tetradecane |
| 15 | 15.591 | 15.495 | 16.075 | 624789 | 0.11 | 48505 | 0.04 | 12.88 | MI | Hexadecane |
| 16 | 18.304 | 18.240 | 18.525 | 309273 | 0.06 | 40715 | 0.03 | 7.60 | MI | Octadecane |
| 17 | 20.747 | 20.690 | 20.940 | 717034 | 0.13 | 133420 | 0.10 | 5.37 | MI | Eicosane |
| 18 | 23.061 | 23.010 | 23.185 | 1455536 | 0.26 | 332463 | 0.24 | 4.38 | | Docosane |
| 19 | 25.244 | 25.195 | 25.345 | 1608958 | 0.29 | 442910 | 0.32 | 3.63 | | Tetracosane |
| | | | | 550896023 | 100.00 | 137811420 | 100.00 | | | |

Appendix E – 1-10: JMP output - Effects tests by volatile compound.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 1.7664e+13 | 108.0077 | <.0001* |
| Water | 1 | 1 | 437204402 | 0.0053 | 0.9420 |
| Vine Tea*Water | 2 | 2 | 6.5263e+11 | 3.9905 | 0.0252* |

Figure E1 – Effects tests for β-cyclocitral.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 4.2125e+15 | 647.4804 | <.0001* |
| Water | 1 | 1 | 5.7251e+13 | 17.5994 | 0.0001* |
| Vine Tea*Water | 2 | 2 | 4.7938e+13 | 7.3682 | 0.0016* |

Figure E2 – Effects tests for 1-penten-3-ol.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 8.8114e+14 | 353.0500 | <.0001* |
| Water | 1 | 1 | 1.2267e+14 | 98.3039 | <.0001* |
| Vine Tea*Water | 2 | 2 | 1.9766e+14 | 79.1958 | <.0001* |

Figure E3 – Effects tests for (E,Z)-2,4-heptadienal.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 7.2657e+14 | 533.9149 | <.0001* |
| Water | 1 | 1 | 1.0907e+14 | 160.3014 | <.0001* |
| Vine Tea*Water | 2 | 2 | 1.5476e+14 | 113.7231 | <.0001* |

Figure E4 – Effects tests for (E,E)-2,4-heptadienal.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 6.0971e+14 | 452.5818 | <.0001* |
| Water | 1 | 1 | 8.2385e+13 | 122.3069 | <.0001* |
| Vine Tea*Water | 2 | 2 | 2.0964e+13 | 15.5617 | <.0001* |

Figure E5 – Effects tests for (E)-2-hexenal.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 4.6283e+13 | 263.1832 | <.0001* |
| Water | 1 | 1 | 1.6364e+12 | 18.6103 | <.0001* |
| Vine Tea*Water | 2 | 2 | 2.2533e+12 | 12.8131 | <.0001* |

Figure E6 – Effects tests for (Z)-4-heptenal.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 3.3725e+15 | 1162.336 | <.0001* |
| Water | 1 | 1 | 3.1125e+13 | 21.4550 | <.0001* |
| Vine Tea*Water | 2 | 2 | 2.9826e+13 | 10.2798 | 0.0002* |

Figure E7 – Effects tests for 6-methyl-5-hepten-2-one.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 3.7288e+15 | 1004.229 | <.0001* |
| Water | 1 | 1 | 9.1396e+13 | 49.2295 | <.0001* |
| Vine Tea*Water | 2 | 2 | 8.7821e+13 | 23.6517 | <.0001* |

Figure E8 – Effects tests for hexanal.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 1.9353e+12 | 13.7047 | <.0001* |
| Water | 1 | 1 | 1.3802e+13 | 195.4723 | <.0001* |
| Vine Tea*Water | 2 | 2 | 2.3255e+12 | 16.4676 | <.0001* |

Figure E9 – Effects tests for nonanal.

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Vine Tea | 2 | 2 | 8.0804e+10 | 1.2089 | 0.3078 |
| Water | 1 | 1 | 9.4567e+11 | 28.2953 | <.0001* |
| Vine Tea*Water | 2 | 2 | 1.9063e+11 | 2.8519 | 0.0680 |

Figure E10 – Effects tests for β-ionone.

Appendix F – 1-10: JMP output - Pairwise comparisons of least squares means using the Tukey-Kramer HSD (Honestly Significant Difference) test by volatile compound.

| Level | | | | Least Sq Mean |
|---|---|---|---|---|
| B,tap | A | | | 2121347.9 |
| B,DI | A | | | 1850156.3 |
| A,tap | | B | | 1410173.6 |
| A,DI | | B | | 1388499.8 |
| C,DI | | | C | 705500.0 |
| C,tap | | | C | 430059.1 |

Levels not connected by same letter are significantly different.

Figure F1 – LS means differences Tukey HSD for β-cyclocitral.

| Level | | | | | Least Sq Mean |
|---|---|---|---|---|---|
| B,tap | A | | | | 23057007 |
| B,DI | | B | | | 18382660 |
| A,tap | | | C | | 4671569 |
| A,DI | | | C | | 3475178 |
| C,tap | | | | D | 582965 |
| C,DI | | | | D | 275729 |

Levels not connected by same letter are significantly different.

Figure F2 – LS means differences Tukey HSD for 1-penten-3-ol.

| Level | | | | Least Sq Mean |
|---|---|---|---|---|
| B,DI | A | | | 13527929 |
| B,tap | | B | | 5104233 |
| A,DI | | | C | 1111366 |
| C,DI | | | C | 699579 |
| A,tap | | | C | 672429 |
| C,tap | | | C | 518888 |

Levels not connected by same letter are significantly different.

Figure F3 – LS means differences Tukey HSD for (E,Z)-2,4-heptadienal.

| Level | | | | Least Sq Mean |
|---|---|---|---|---|
| B,DI | A | | | 12259951 |
| B,tap | | B | | 4639513 |
| A,DI | | | C | 1417271 |
| A,tap | | | C | D 692832 |
| C,DI | | | C | D 423565 |
| C,tap | | | | D 241141 |

Levels not connected by same letter are significantly different.

Figure F4 – LS means differences Tukey HSD for (E,E)-2,4-heptadienal.

| Level | | | | | Least Sq Mean |
|---|---|---|---|---|---|
| B,DI | A | | | | 11676632 |
| B,tap | | B | | | 7535177 |
| A,DI | | | C | | 4088072 |
| C,DI | | | | D | 2607199 |
| A,tap | | | | D | E 1968598 |
| C,tap | | | | | E 1457098 |

Levels not connected by same letter are significantly different.

Figure F5 – LS means differences Tukey HSD for (E)-2-hexenal.

| Level | | | | Least Sq Mean |
|---|---|---|---|---|
| B,DI | A | | | 2636500.8 |
| B,tap | | B | | 1704060.9 |
| A,DI | | | C | 312366.9 |
| A,tap | | | C | 232390.8 |
| C,DI | | | C | 145704.8 |
| C,tap | | | C | 102819.6 |

Levels not connected by same letter are significantly different.

Figure F6 – LS means differences Tukey HSD for (Z)-4-heptenal.

| Level | | | | Least Sq Mean |
|---|---|---|---|---|
| B,DI | A | | | 19744868 |
| B,tap | | B | | 16124388 |
| A,DI | | | C | 1633767 |
| C,DI | | | C | 1183597 |
| A,tap | | | C | 1166951 |
| C,tap | | | C | 715657 |

Levels not connected by same letter are significantly different.

Figure F7 – LS means differences Tukey HSD for 6-methyl-5-hepten-2-one.

| Level | | | | | Least Sq Mean |
|---|---|---|---|---|---|
| B,DI | A | | | | 24150158 |
| B,tap | | B | | | 18291327 |
| A,DI | | | C | | 8279161 |
| A,tap | | | | D | 5963136 |
| C,tap | | | | E | 1641955 |
| C,DI | | | | E | 1272929 |

Levels not connected by same letter are significantly different.

Figure F8 – LS means differences Tukey HSD for hexanal.

| Level | | | | Least Sq Mean |
|---|---|---|---|---|
| B,DI | A | | | 2197679.7 |
| A,DI | | B | | 1503131.4 |
| C,DI | | B | | 1214669.6 |
| A,tap | | | C | 605791.8 |
| C,tap | | | C | 595048.8 |
| B,tap | | | C | 557430.6 |

Levels not connected by same letter are significantly different.

Figure F9 – LS means differences Tukey HSD for nonanal.

| Level | | | | Least Sq Mean |
|---|---|---|---|---|
| B,DI | A | | | 1024888.8 |
| C,DI | A | | | 1001835.4 |
| A,DI | A | B | | 973232.1 |
| A,tap | A | B | C | 851110.0 |
| C,tap | | B | C | 731252.8 |
| B,tap | | | C | 607213.3 |

Levels not connected by same letter are significantly different.

Figure F10 – LS means differences Tukey HSD for β-ionone.

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