Sensory attributes and volatile compounds characteristics of *Zanthoxylum* genus.

by

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Abstract

A lexicon was developed to describe the aroma and flavor profiles of sensory characteristics of *Zanthoxylum bungeanum*, also known as Sichuan pepper. As a further investigation, an instrumental analysis was conducted in order to identify the volatile compounds of Sichuan pepper.

For the sensory lexicon, a highly trained descriptive analysis panel was used to characterize the aroma and flavor characteristics for Sichuan Pepper. A total of 32 sensory attributes were detected which included 15 attributes for aroma profile and 17 attributes for flavor profile. The aroma profile included peppery, green, citrus, pungent, fennel anise, brothy, floral perfumery, piney, woody, fruity, sweet aromatics, petroleum-like, chive, earthy, brown, smoke and menthol. The flavor profile included green, citrus, minty, fruity, pepper, petroleum-like, floral, cardboard, green viney, pungent, umami, earthy, piney, sweet, bitter, astringent, tingle, and numbing. The lexicon was used to characterize 16 different Sichuan pepper, including subspecies, locations, and brands, which showed that the lexicon could effectively differentiate among samples. Differences in the peppers were analyzed by analysis of variance (ANOVA) and principal components analysis.

Gas chromatography-mass spectrometry (GC-MS) associated with a modified headspace solid-phase microextraction was used for to characterize the volatile fractions of the 16 Sichuan Pepper samples to differentiate green and red Sichuan peppers. A total of 99 volatile compounds were tentatively detected among 16 samples. The 46 terpenoid compounds detected were the most abundant volatile compounds. Partial least squares regression was used to associate descriptive sensory characteristics to volatile compounds. Possible relationships between volatile compounds and sensory attributes were detected, in which the most prominent associations

included β-Copaene related to Anise/Fennel aroma and Fruity flavor,α-Phellandrene related to Earthy aroma, cis-Carveol and 2-Phenylethyl acetate related to Perfumery/Floral aroma, Sabinene hydrate related to Woody aroma and Petroleum-Like flavor, (+)-β-Thujone related to Anise/Fennel aroma, Fruity aroma, Woody aroma, Green flavor, Petroleum-Like flavor, Green Viney flavor, Pungent flavor, and Bitter flavor, β-Pinene related to Citrus flavor, Caryophyllene related to Anise/Fennel aroma, δ-Limonene related to Menthol aroma, Linalyl acetate related to Pepper aroma, Fruity aroma and Citrus flavor, β-Selinene related to Pepper flavor and Petroleum-Like flavor. Results suggested age would be an important factor to differentiate Sichuan peppers.

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Dedication

This is dedicated to my family here and my family all the way in Chengdu. Your support, enthusiasm and love have made this possible in my life. Thanks to my wife and little daughter for always being there for me, working with me for our sweet home here. Thanks to my parents in Chengdu, I'm so glad that I have ever felt as close with you than when I have been so far away since you support me selflessly as you always do!

Chapter 1 - Literature Review

Overview

Sichuan pepper, also known as *Huajiao*, is believed to originate from Sichuan Province, and contributes as a key spice material in Sichuan cuisine. Although the name refers to the plant as a pepper, it's completely different from either black pepper or chili pepper, and belongs to the family Rutaceae, in the *Zanthoxylum* global genus. It has more than 250 different species all over the world, and is widely used in Chinese (especially Sichuan), Japanese, Korean, Nepalese, Bhutanese, Batak Toba (North Sumatra) and Konkani (India) cuisines (Lim, 2012).

Recordings of the use of *Z. bungeanum* Maxim in some ritual activities go back more than 2200 years (Huang et, al., 2006). In traditional Chinese culture, the pericarp of *Zanthoxylum* genus was used as a kind of herbal medicine material for gastralgia and dyspepsia. (Lim, 2012). Also, *Z. bungeanum* was called 'toothache trees' in western folk medicine, because the anesthetic property was useful in the treatment of pain (Bautista et al., 2008). In Asia, the most used *Zanthoxylum* genus are called Sichuan pepper in commercial markets and daily life. Its pericarp can be used as a seasoning for food. The fruits provide an extract oil, widely applied as condiment or seasoning materials in Asian cuisine, but also applied in medical treatment, such as treatment for inflammation and asthma (Zhang et al., 2017; Tang et al., 2014). The strong pungent and floral flavor of Sichuan pepper can help to mask bloody and animal flavor for meat. The dried pericarp of Sichuan pepper is ground and added to the fried food, or mixed with star anise, cassia, clove and fennel as seasoning. The dried seeds can be extracted into oil or processed into soap.

General Characteristics of Sichuan Pepper

In botany

As mentioned above, Sichuan pepper is always referred to as *Zanthoxylum* genus in botany. There are 43 species and 8 variants of *Zanthoxylum* genus in China, which grow in many habitats below 3200m and over 22 different regions and provinces (Wu et al., 2008). Typically, there are five main genera of *Zanthoxylum* used and contributed in daily life. The *Z. bungeanum* Maxim. is in southwest of China and related to many cultivars of Sichuan pepper, such as *Da Hongpao*, and *Hanyuan Huajiao* (Xiang et al., 2016). The *Z. armatum* DC cultivars *Jinyangqing huajiao* and *Tengjiao* is often known as toothache tree which has a characteristic fresh aroma and provides a numbing sensation. The *Z. simulans* Hance is native to eastern China region and be described as myrcene aroma (Chyau et al., 1996). The *Z. piasezkii* Maxim is in the southwest and central part of China and includes eucalyptol in its extracted oil (Zhang et al., 2017). *Z. schinifolium* Sieb et Zucc is native to the north region of China and has a grey green pericarp and white inner core (Zhao et al., 2013).

The *Zanthoxylum* genus is a shrub approximately 3 – 7 m in height and has branched stems around 3 – 8 cm in diameter. The leaves are light green to pale green with 1 – 7 cm in length and 2 – 3 cm in width. Each branch often has 5-13 narrow leaflets; leaflets along the stems are in the opposite position, and often present sessile, ovate, elliptic, or sparsely lanceolate shape. The leaves have a fine cracked tooth shape at the edges of the surface. Some oily spots are scattered on the surface of the leaf edge. The midrib of leaves is slightly sunken on the surface, and the back of the leaf often has reddish-brown inflorescences. The flowers are on top of the lateral branches which have 6 – 8 segments roughly same in shape and size. The color of flowers are yellowish green. (Lim, 2012). Fruits color are green to red. The single fruit valve diameter is 4-5 mm. Micro-protruding oil spots are scattered on the surface of fruit. The seeds of plant are 3.5-4.5 mm in length, and the flowering period is in April to May; fruit harvest period is in August to October, and the seeds are processed by drying under sun or immersed into salt water (Zhang, M et al., 2017; Huang et al., 1997). The fruits have a pungent and numbing sensation odor and taste and may also boost the umami taste (Frerot et al., 2015).

There is considerable research related to Sichuan pepper, but the differentiation of these pepper genus and cultivars might have some confusion. In the market, Sichuan peppers always are divided by its color: red and green. The red pepper includes species of *Z. bungeanum* Maxim, aged *Z. armatum* DC, *Z. simulans* Hance, and *Z. piasezkii* Maxim. The green pepper includes species of fresh *Z. armatum* DC, and *Z. schinifolium* Sieb. et Zucc. There are not only distinguished odor differences between red and green Sichuan peppers, but also differences in chemical compounds. (Yang et al., 2008; Xiang et al., 2016; Perichet et al., 2018). The spicy, tingling, and numbing flavors are the main characteristics for Sichuan Pepper. Different environmental conditions can have different effects on harvest of the *Zanthoxylum* genus (Xiang et al., 2016) indicated temperature is related to the volatile oil content for *Z. armatum*, and precipitation is related to the non-volatile ethers in *Z. bungeanum*.

According to People's Republic of China Forestry Industry Standard, code of LY/T1652 — 2005, the standard quality level for Sichuan pepper is graded by physical, chemical and, in-part, its sensory properties. The physical and chemical indicators include volatile oil content, moisture and solid impurities. The sensory aspects include color, general odor, taste, hand feeling, and appearance of fruit-shape particles. However, in this standard there is no method to determine the characteristics of flavor or the contribution of numbing/tingling substances. This quality control standard focuses on grading shelf-life of Sichuan pepper fruit, but is not focused on differentiating types of Sichuan pepper. To detect and identify the differences among these Sichuan pepper genus in a systematic method needs more effort in future research. Table 1 highlights information on different commercial species.

Table 1.1 Different species contribution to the commercial product and the main characteristics of their properties

Color	Commercial Name	Genus	Cultivars	Harvest Age
Red	Red Sichuan Pepper (*Huajiao*)	*Zanthoxylum bungeanum* Maxim	*Da Hongpao,* *Hanyuan Huajiao, Yuexigong Jiao, Lingshan Zhenglujiao*	Middle Fall, Matured Fruit
Red	Red Sichuan Pepper (*Huajiao*)	*Zanthoxylum armatum* DC	*Jinyangqing huajiao*	Middle Fall, Matured Fruit
Red	Red Sichuan Pepper (*Huajiao*)	*Z. simulans* Hance	*Ye Huajiao*	Fall, Matured Fruit
Red	Red Sichuan Pepper (*Huajiao*)	*Z. piasezkii* Maxim	*Dajing Huajiao,* *Chuanshan Huajiao*	Fall, Matured Fruit
Green	Green Sichuan Pepper (*Qinghuajiao*)	*Zanthoxylum armatum* DC	*Jinyangqing huajiao*	End Summer, Prematured Fruit
Green	(*Tengjiao*)	*Zanthoxylum armatum* DC	*Hongya Tengjiao*	End Summer, Prematured Fruit
Green	Green Sichuan Pepper (*Qingjiao*)	*Zanthoxylum schinifolium* Sieb. et Zucc	*Ya jiao,* *Xiangjiaozi*	Fall Term, Matured Fruit

Applications of Sichuan peppers

Phytochemistry

It has been reported that the essential oil of *Zanthoxylum bungeanum* helps to permeate cells and promote penetration of transdermal drugs (Lan et al., 2014) more than individual compounds alone. For example, permeation capacities were terpinene-4-ol≈1,8-cineole < limonene < *Z. bungeanum* oil.

The main flavor characteristics of Sichuan Pepper are derived from the volatile oil. The content of volatile oil is one of the main factors to identify the chemical and physical properties (Bhatt et al., 2017). These volatile oils contain many small molecular substances such as olefins, ketones, alcohols, esters, and epoxies (Chyau et al., 1996; Zhang et al., 2017). The chemical constituents and contents of the volatile oil of *Zanthoxylum bungeanum* Maxim are affected by many factors such as variety, origin and climate. Chen and others (2015) analyzed the composition of volatile oil of red Sichuan Pepper from eight different regions in Gansu, Shaanxi and Sichuan provinces. It was found that the volatile oils of red peppers in different regions were different. The volatile oil components were different among samples, and the amount of the same chemical components also was different among samples.

Alkylamides

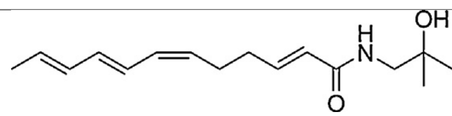
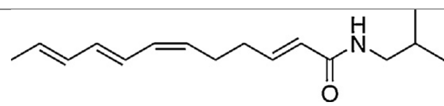
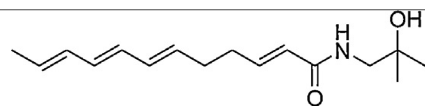
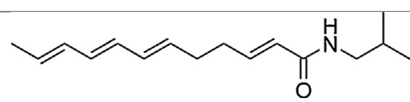
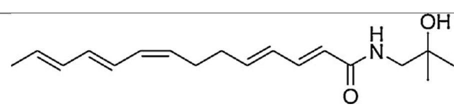
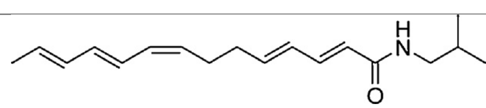
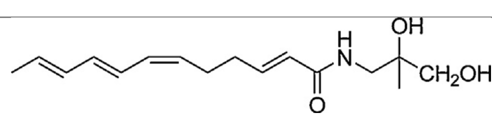
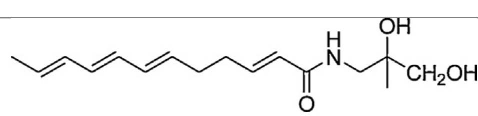
The category of of alkylamides were an essential part of *Zanthoxlum* genus, which provided abundant pungent and numbing sensations. Hydroxy-α-sanshool is the most abundant alkylamide in the *Zanthoxylum* genus and plays a major role in sensory properties. The α-sanshool, γ-sanshool and hydroxy γ-sanshool compounds were both identified in samples and contributed with hydroxy-α-sanshool as 4 principle components in the *Zanthoxylum* genus

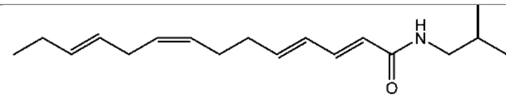
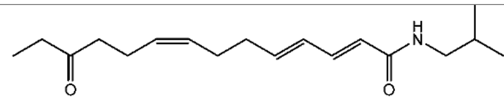
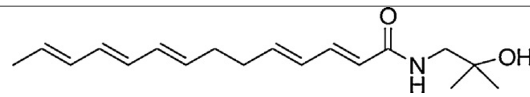
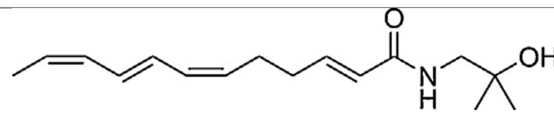
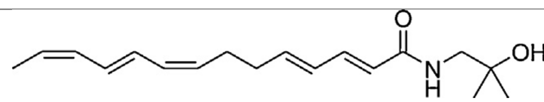
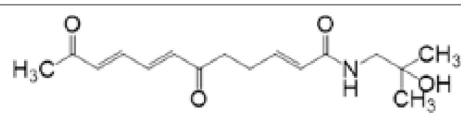
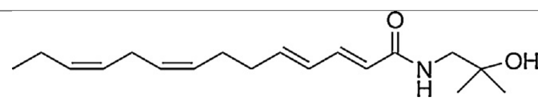
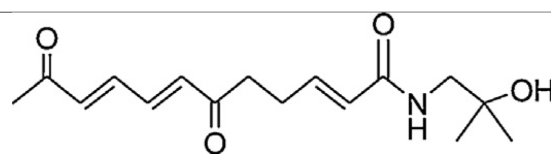
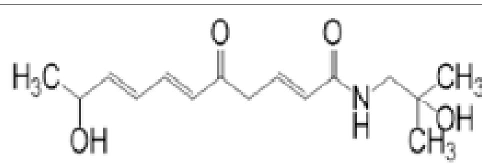
(Yasuda et al., 1982). Over 40 alkylamides have been detected in different *Zanthoxylum* genus and are shown in Table 1.2. Different alkylamides, hydroxyl-γ-sanshool and bungeanool have been shown to distinguish *Zanthoxylum bungeanum* and *Zanthoxylum schinifolium*, known as red Sichuan pepper and green Sichuan pepper respectively.

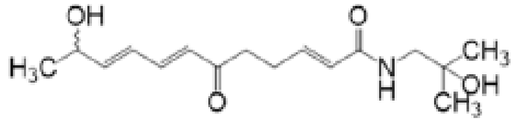
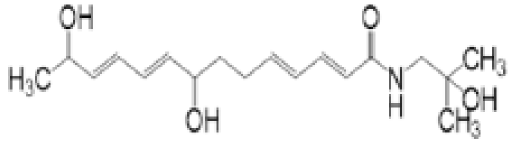
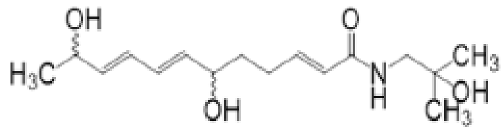
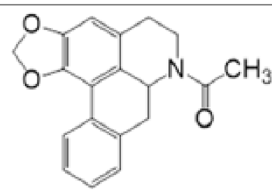
The β-Sanshool and hydroxy-β-Sanshool also are key compounds in the *Zanthoxylum* genus oils. However, these two chemicals are reasonably tasteless and provide only a little bitter flavor (Mizutani et al., 1988). Specifically, hydroxy-β-Sanshool couldn't be detected in human taste tests at the 100 μg level (Yang., 2008). Similarly, (2E,4E,8E,10E,12E)-20-hydroxy-N-isobutyl-2,4,8,10,12-tetradecatetraenamide, the all-trans alkylamides, were tasteless. That compares to the amides with a cis double bond, such as hydroxy-α-sanshool, hydroxyl-ε-sanshool and hydroxy γ-sanshool, which were strongly pungent (Mizutani et al., 1988; Bader et al., 2014).

(2E,7E,9E)-N-(2-hydroxy-2-methylpropyl)-6,11-dioxo-2,7,9-dodecatrienamide and (2E,6E,8E)-N-(2-hydroxy-2-methylpropyl)-10-hydroxy-5-oxo-undeca-2,6,8-trienamide were identified and isolated from the pericarps of *Zanthoxylum bungeanum* (Tian et al., 2016). One-dimensional and two-dimensional nuclear magnetic resonance spectroscopy (NMR) analysis was applied to identify the structure of these isobutylhydroxyamides.

Table 1.2 Chemical Structure of Alkylamides in Zanthoxylum genus

Name	Chemical Structure	Reference
Hydroxy-α-sanshool		(Bader et al., 2014)
α-sanshool		(Yang, 2008)
Hydroxy-β-sanshool		(Bader et al., 2014)
β-Sanshool		(Yang, 2008)
Hydroxyl-γ-sanshool		(Bader et al., 2014)
γ-Sanshool		(Yang, 2008)
Dihydroxy α-sanshool		(Ji et al., 2019)
Dihydroxy β-sanshool		(Ji et al., 2019)

Dihydro-γ-sanshool		(Yang, 2008)
Dihydrobungeanool		(Yang, 2008)
Hydroxy-γ-isosanshool		(Bader et al., 2014)
Hydroxyl-ε-sanshool		(Bader et al., 2014)
Hydroxyl-ζ-sanshool		(Bader et al., 2014)
Bugeanumamide A		(Wang et al., 2016)
Bungeanool		(Wang et al., 2016)
(2E,7E,9E)-N-(2-hydroxy-2-methylpropyl)- 6,11-dioxo-2,7,9-dodecatrienamide		(Tian et al., 2016)
(2E,6E,8E)-N-(2-hydroxy-2-methylpropyl)-10-hydroxy-5-oxo-undeca-2,6,8-trienamide		(Tian et al., 2016)

(11RS)-(2E,7E,9E)-11-hydroxy-N-(2-hydroxy-2-methylpropyl)-6-oxo-2,7,9-dodecatrienamide		(Wang et al., 2016)
(10RS,11RS)-(2E,6Z,8E)-10,11-Dihydroxy-N-(2-hydroxy-2-methylpropyl)-2,6,8-dodecatrienamide		(Wang et al., 2016)
(6RS,11RS)-(2E,7E,9E)-N-(2-hydroxy-2-methylpropyl)-6,11-dioxo-2,7,9-dodecatrienamide		(Wang et al., 2016)
(2E,4E,8E,10E,12E)-20-hydroxy-N-isobutyl-2,4,8,10,12-tetradecatetraenamide		(Mizutani et al., 1988)

Volatiles

Besides the non-volatile compounds, a considerable number of volatile compounds differentiated among ages of the *Zanthoxylum* genus (Table 1.3). The major components of red Sichuan pepper were linalyl acetate (15%), linalool (13%), and limonene (12%), which were differentiated to green Sichuan pepper linalool (29%), limonene (14%), and sabinene (13%) (Yang., 2008). This research also extrapolated the major aroma character impact by volatiles through the threshold value of a component. It showed α-terpineol, geraniol, and 1,8-cineole played important role in flavor of Sichuan pepper, although the percentage of components were not high.

Table 1.3 Volatile compounds found in different aged Zanthoxylum genus

Chemical Names	Green	Red	Reference
1,8-cineole	×	×	(Yang, 2008)
10-epi-γ-eudesmol	×	×	(Yang, 2008)
1-butanol	×	nd	(Yang, 2008)
1-heptanol	×	nd	(Yang, 2008)
1-hexanol	×	nd	(Yang, 2008)
1-nonanol	×	nd	(Yang, 2008)
1-terpineol	×	×	(Yang, 2008)
2,3-dehydro-1,8-cineole	nd	×	(Yang, 2008)
2,4-diethylheptan-1-ol	×	nd	(Yang, 2008)
2,4-dimethylacetophenone	×	×	(Yang, 2008)
2,4-heptadienal	nd	×	(Yang, 2008)
2,4-hexadienal	nd	×	(Yang, 2008)
2,6-dimethyl-2-heptenal	×	nd	(Yang, 2008)
2,6-dimethylheptanal	×	nd	(Yang, 2008)
2-methyl-3-buten-2-ol	×	×	(Yang, 2008)
2-oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, 6-acetate	×	×	(Yang, 2008)
2-phenylethyl acetate	×	×	(Yang, 2008)
2-propenal	×	nd	(Yang, 2008)
2-propenoic acid, 3-phenyl-, methyl ester	×	nd	(Yang, 2008)
2-tridecanone	×	nd	(Yang, 2008)
3,6-α-faαnesene	×	×	(Yang, 2008)
3-buten-2-one	×	×	(Yang, 2008)
3-methylpentadecane	×	nd	(Yang, 2008)
3-nonanone	nd	×	(Yang, 2008)

4-methyl-1-heptanol	×	nd	(Yang, 2008)
4-methyl-1-pentanol	×	nd	(Yang, 2008)
4-terpinenol	×	×	(Yang, 2008)
4-terpinenyl acetate	×	×	(Yang, 2008)
6-methyl-1-heptanol	×	nd	(Yang, 2008)
6-methyl-5-hepten-2-one	nd	×	(Yang, 2008)
6-methylheptanal	×	nd	(Yang, 2008)
acetaldehyde	×	×	(Yang, 2008)
allo-aromadendrene	nd	×	(Yang, 2008)
benzeneacetic acid, methyl ester	×	nd	(Yang, 2008)
bicyclogermacrene	×	nd	(Yang, 2008)
bois de rose ketone	nd	×	(Yang, 2008)
borneol	×	nd	(Yang, 2008)
bornyl acetate	×	×	(Yang, 2008)
butyl butyrate	×	nd	(Yang, 2008)
camphene	×	×	(Yang, 2008) (Gong et al., 2009)
carvone	×	×	(Yang, 2008)
caryophyllene oxide	×	×	(Yang, 2008) (Zhang et al., 2016)
cis-carveol	×	×	(Yang, 2008)
cis-carveyl acetate	nd	×	(Yang, 2008)
cis-linalool oxide	×	×	(Yang, 2008)
cis-linalool oxide	×	×	(Yang, 2008)
cis-*p*-2-menthen-1-ol	×	×	(Yang, 2008)
cis-*p*-mentha-2,8-dien-1-ol	n.d.	×	(Yang, 2008)
cis-sabinene hydrate	×	nd	(Yang, 2008)

cis-β-ocimene	×	×	(Yang, 2008)
citronellal	×	×	(Yang, 2008)
citronellol	×	nd	(Yang, 2008)
citronellyl acetate	nd	×	(Yang, 2008)
crotonaldehyde	×	nd	(Yang, 2008)
cryptone	nd	×	(Yang, 2008)
decanal	×	nd	(Yang, 2008)
decane	×	nd	(Yang, 2008)
diacetyl	×	nd	(Yang, 2008)
dihydrocarvone	×	×	(Yang, 2008)
epi-α-cadinol	×	×	(Yang, 2008)
ethyl pyruvate	nd	×	(Yang, 2008)
geraniol	×	×	(Yang, 2008)
geranyl acetate	nd	×	(Yang, 2008)
germacrene B	nd	×	(Yang, 2008)
germacrene D	×	×	(Yang, 2008)
germacrene D-4-ol	×	×	(Yang, 2008)
heptyl acetate	×	×	(Yang, 2008)
hexadecane	×	nd	(Yang, 2008)
hexanal	×	×	(Yang, 2008)
hexyl acetate	×	×	(Yang, 2008)
hotrienol	×	×	(Yang, 2008)
humulene oxide	nd	×	(Yang, 2008)
isoamyl alcohol	×	×	(Yang, 2008)
isobutanol	×	×	(Yang, 2008)
isobutyl acetate	×	×	(Yang, 2008)
isodecanal	×	nd	(Yang, 2008)

isopiperitenone	nd	×	(Yang, 2008)
isopulegol	nd	×	(Yang, 2008)
lilac aldehyde	nd	×	(Yang, 2008)
limonene	×	×	(Yang, 2008)
linalool	×	×	(Yang, 2008)
linalool oxide dehydrate	nd	×	(Yang, 2008)
linalyl acetate	×	×	(Yang, 2008)
linalyl formate	×	×	(Yang, 2008)
linalyl propionate	nd	×	(Yang, 2008)
methyl 4-methylvalerate	×	×	(Yang, 2008)
methyl citronellate	×	nd	(Yang, 2008)
myrcene	×	×	(Yang, 2008)
myrcene epoxide	×	×	(Yang, 2008)
myrtenal	×	×	(Yang, 2008)
myrtenol	×	×	(Yang, 2008)
neoiso-isopulegol	×	nd	(Yang, 2008)
nerol	×	×	(Yang, 2008) (Zhang et al., 2016)
nerol oxide	nd	×	(Yang, 2008)
neryl acetate	×	×	(Yang, 2008)
nonanal	×	×	(Yang, 2008)
octanal	×	×	(Yang, 2008)
p,α-dimethylstyαene	×	×	(Yang, 2008)
p-1,8-menthadienyl-7 acetate	nd	×	(Yang, 2008)
p-cymen-8-ol	×	×	(Yang, 2008)
p-cymene	×	×	(Yang, 2008) (Li et al., 2014)

pentadecane	×	nd	(Yang, 2008)
perillene	×	×	(Yang, 2008)
pinocarvone	×	×	(Yang, 2008)
p-isopropylbenzyl alcohol	nd	×	(Yang, 2008)
p-menth-1-en-9-al	×	×	(Yang, 2008)
p-mentha-1(7),2,8-trien	nd	×	(Yang, 2008)
p-mentha-1,3,8-triene	×	×	(Yang, 2008)
p-mentha-1,8(10)-dien-9-ol	nd	×	(Yang, 2008)
p-mentha-1,8-dien-4-ol	×	×	(Yang, 2008)
prenol	nd	×	(Yang, 2008)
rosefuran	nd	×	(Yang, 2008)
sabinene	×	×	(Yang, 2008) (Gong et al., 2009)
spathulenol	×	nd	(Yang, 2008)
terpinolene	×	×	(Yang, 2008)
tetradecane	×	×	(Yang, 2008)
toluene	×	×	(Yang, 2008)
trans-allo-ocimene	×	×	(Yang, 2008)
trans-carveol	×	×	(Yang, 2008)
trans-limonene oxide	×	nd	(Yang, 2008)
trans-linalool oxide	×	×	(Yang, 2008)
trans-myrtanol	×	nd	(Yang, 2008)
trans-nerolidol	×	×	(Yang, 2008)
trans-ocimene epoxide	nd	×	(Yang, 2008)
trans-pinocarveol	×	×	(Yang, 2008)
trans-piperitol	nd	×	(Yang, 2008)
trans-*p*-mentha-2,8-dien-1-ol	nd	×	(Yang, 2008)

trans-sabinene hydrate	×	×	(Yang, 2008)
trans-sabinene hydrate acetate	nd	×	(Yang, 2008)
trans-β-farnesene	nd	×	(Yang, 2008)
verbenol	×	nd	(Yang, 2008)
α-beαgamotene	nd	×	(Yang, 2008)
α-cadinol	×	nd	(Yang, 2008) (Gong et al., 2009)
α-calacoαene	nd	×	(Yang, 2008)
α-copaene	nd	×	(Yang, 2008)
α-cubebene	nd	×	(Yang, 2008)
α-elemol	×	nd	(Yang, 2008)
α-farnesene	nd	×	(Yang, 2008)
α-humulene	×	×	(Yang, 2008)
α-muurolene	×	nd	(Yang, 2008)
α-phellandrene	×	×	(Yang, 2008)
α-pinene	×	×	(Yang, 2008)
α-selinene	×	nd	(Yang, 2008)
α-terpinene	×	×	(Li et al., 2014)
α-terpineol	×	×	(Yang, 2008) (Li et al., 2014)
α-terpinyl acetate	×	×	(Yang, 2008) (Gong et al., 2009)
α-thujene	×	×	(Yang, 2008)
α-thujone	×	×	(Yang, 2008)
β-caryophyllene	×	×	(Yang, 2008) (Gong et al., 2009)
β-cubebene	nd	×	(Yang, 2008)

			(Gong et al., 2009)
β-elemene	nd	×	(Yang, 2008)
β-phellandrene	×	×	(Yang, 2008) (Gong et al., 2009)
β-pinene	×	×	(Yang, 2008)
β-thujone	×	×	(Wang et al., 2010)
γ-cadinene	×	×	(Yang, 2008)
γ-gurjunene	nd	×	(Yang, 2008)
γ-terpinene	×	×	(Yang, 2008)
δ-3-carene	×	nd	(Yang, 2008)
δ-cadinene	×	×	(Yang, 2008)
δ-cadinol	×	nd	(Yang, 2008)

nd - Not Detected. x - Detected

Flavor

The flavor characteristics of Sichuan pepper are characterized as tingling, numbing, astringent, and bitter (Sugai et al.,2005). The tingling sensation of Sichuan pepper was deteremined to be similar to a vibration around 50 Hz frequency on the tongue and lips (Hagura et al., 2013). Zhang and others (2017) generated pungency terms showing the mixed sensation of numbing, tingling, astringent, and vibrating sensation from sanshools and hydroxyl sanshools.

When Sichuan pepper is used in food, the food would promote the secretion of saliva and increase appetite. Frerot et al. (2015) found and illustrated the umami amide in *Zanthoxylum* piperitum. It provided new utilization of Sichuan pepper as a potential flavor enhancer. Ding and others (2017) showed the bitter taste would relate to the alcohol and ketone families of chemical components. However, little researches reported the association for Sichuan pepper samples between general sensory profiles and chemical components.

Pharmacology

Antioxidants Contents

In food systems, Zanthoxylum *bungeanum* showed inhibitory effects on the formation of heterocyclic amines to grilled beef patties (Zeng et al., 2018). Heterocyclic amines were the main groups of carcinogenic compounds formed by processing protein rich foods at high temperature, and had potential to cause cancer (Jinap et al., 2015; Szterk et al., 2015). Also, *Zanthoxylum bungeanum* is used as a daily antioxidant intake for some people, although it contributed only a small part to the food dishes. The extracted oil of *Zanthoxylum* genus had abundant chlorogenic acid, rutin and several phenolic compounds, which made the extracted oil to be an economical natural antioxidant seasoning (Lu et al., 2011).

Leaves contain abundant flavonoids that may have prominent antioxidant abilities. Through high performance liquid chromatography coupled with tandem mass spectrometry with electrospray ionization and negative ion detection (HPLC-ESI-MS/MS), Yang et al., (2013) reported that eight flavonoids were identified in leaves of *Zanthoxlum bungeanum* maximum. These polyphenols performed well in reducing the reactive oxygen species (ROS) in HT-29 cells. Zhang et al., (2014) reported that nine flavonoids were detected by Electrospray ion trap mass spectrometry (ESI-MS) and Nuclear magnetic resonance spectra (NMR). All nine flavonoids were isolated from leaves of *Zanthoxylum bungeanum* and tested in vitro and in vivo antioxidant activities. Results also showed good antioxidant by these flavonoids.

The extraction oil from *Zanthoxlum* genus conducted to be a supplementation dietary treatment for ulcerative colitis (Zhang et al., 2017).

Effects on Sensory Systems

The *Zanthoxylum* genus has an impact on sensory systems and is described in many ways. The term of general 'pungent flavor' was applied to describe the main sensory property of *Zanthoxylum* genus. A tingling and numbing sensation were the key component of pungent flavor, and many researchers have tried to uncover the sensory mechanism of this special sensation.

Hydroxy-α-sanshool from the pericarp of *Zanthoxylum* genus is the main chemical responsible for the numbing and tingling sensation (Yasuda et al., 1982; Mizutani et al., 1988; Yang, 2008; Gong et al., 2009). It has cis- double bond to evoke its unique tingling sensation. However, various mechanism of hydroxy-α-sanshool were claimed. The sensory neurons of TRPV1 and TRPA1 were found to relate to the tingling sensation (Koo et al., 2007). Hydroxy-α-sanshool could affect TRPV1 or TRPA1 by causing the Ca^{2+} influx. Sugai et al., (2005) also found sanshools could activate TRPA1. However, they still mentioned their potency was very weak. Another view suggests that hydroxy-α-sanshool could excite somatosensory neurons by inhibiting two-pore potassium channels (KCNK3, KCNK9 and KCNK18) (Bautista et al., 2008). This provides an anesthetic or counter-irritant sensation called numbing.

Summary and Research Objectives

None of the research found on Sichuan Pepper has studied flavor aspects in depth. Beyond umami, bitter and numbing or pungency, little information exists on the flavor characteristics of these peppers. Further, little research was found on the association of sensory and volatile compounds in these peppers. The objectives of this thesis were to a) understand what flavor characteristics does Sichuan Pepper provide, b) examine how is the flavor affected by the species, age, or place of origin, and c) to determine what volatile chemical compounds may be associated with those additional flavors beyond the numbing sensation.

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Chapter 2 - Flavor Lexicon Development and Descriptive Analysis of Subspecies of Sichuan Pepper

Abstract

A lexicon was developed to describe the aroma and flavor profiles of sensory characteristics of Zanthoxylum bungeanum, also known as Sichuan pepper. A trained, experienced descriptive analysis panel was used to determine the flavor characteristics for Sichuan Pepper. A total of 32 sensory attributes were detected, which included 15 attributes for aroma and 17 attributes for flavor. The aroma profile included peppery, green, citrus, pungent, fennel anise, brothy, floral/perfumy, piney , fruity, sweet aromatics, petroleum-like, chive, earthy, brown, and menthol. The flavor profile included peppery, green, citrus, pungent, minty, floral, piney, fruity, petroleum-like, earthy, cardboard, green-viney, umami, bitter, astringent, tingle, and numbing. The lexicon was validated and demonstrated through 16 different Sichuan pepper samples, including subspecies, locations, and brands. The differences were analyzed by analysis of variance (ANOVA) and principal components analysis.

Practical Applications

Sichuan pepper is a major flavor ingredient in some types of Asian cooking. A lexicon is needed to identify the effects of different subspecies and processing technologies for Sichuan pepper in order to understand differences in aroma and flavor among these “peppers”. The developed lexicon has been shown to differentiate among samples.

Introduction

Sichuan Pepper, also known as HuaJiao (花椒), is a typical spice in China’s cuisines and famous in provinces located in the southwest and central south regions of China, such as Sichuan Province, Chongqing Province, Shanxi Province and Hunan Province. This plant also is widely distributed in other Asian countries such as Japan, Korean, India, Thailand, and Vietnam etc. Despite its name, Sichuan pepper is neither like Black pepper nor like Chili pepper based on their global genus species. It is related to the global genus Zanthoxylum, and the majority come from the subspecies Zanthoxylum Simulans and Zanthoxylum Bungeanum. The Zanthoxylum genus (Rutaceae) consists of more than 246 species worldwide, including 42 species and 14 varieties in China. The fruit of Zanthoxylum bungeanum is the most popular commercial product in the genus Zanthoxylum and is largely used as a popular condiment in various dishes. So far, multiple varieties of Sichuan Pepper have been cultivated, such as Da Hongpao and Hanyuan Huajiao (Wang et al., 2011; Xiang et al., 2016). The fruits of the Zanthoxylum genus are separated by color and marketed as either Red Sichuan Pepper or Green Sichuan pepper. Plant age is one the important factor that results in color differences with older plants (i.e. 1 vs 5 years of age) producing the red colored peppers. In addition harvest times vary with Green Sichuan Pepper normally harvested in June and Red Sichuan Pepper always harvested in August (Liu et al., 2006). The outer layer of Red Sichuan Pepper fruits is red or purplish-red with scattered warty oil dots. The inner surface of Red Sichuan Pepper fruits is yellowish. The seeds of Red Sichuan Pepper fruits are black color and globular shape and are removed during processing. The outer layer of the Green Sichuan Pepper fruit is green or light green also with scattered warty oil dots. The inner surface of Green Sichuan Pepper fruits is chartreuse to light yellow (Huang et al., 1997). In cuisine application, Red Sichuan Pepper is almost always fried with meat in oil and

Green Sichuan Pepper is typically added into soups and “hot pot”, a typical Chinese dish where meat, fish, and vegetables are cooked in broth and often eaten as individual ingredients.

Considerable research related to Sichuan Pepper’s flavor chemistry has been conducted. Based on previous studies, over 140 compounds have been isolated and identified from Zanthoxylum bungeanum, including alkaloids, terpenoids, free fatty acids and flavonoids (Xiong et al., 1997; Yang, 2008; Xia et al., 2011; Yang et al., 2013). Many methods have been designed to measure and detect the relationship between chemicals and flavors of Sichuan Pepper. The aroma character impact value (AIC) was applied to evaluate the ratio of the concentration of a volatile component in its odor threshold value. The hydroxy-sanshool compounds showed the greatest impact on tingling sensation as well as pungency by inducing tactile sensory fibers and silent fibers which lead to limited sensation of innocuous stimuli (Yang, 2008). Hydroxy-sanshool compounds are important to “pungent” flavor and tingling (Zhang et al. 2019). Chemicals other than hydroxy–sanshool can present both pungent and tingling sensation, such as piperine, fatty acid amides and unsaturated alkylamides with three double bonds (Dawid et al. 2012). The chemical compounds associated with bitter tastes were identified as from the alcohol and ketone families of compounds (Ding et al., 2017).

Given the complexity of chemical compounds, it is difficult to identify and discriminant Sichuan pepper using only ‘pungent’, ‘tingling’, and ‘numbing/anesthetic’ sensory terms. More clarification and a specific lexicon or attributes can provide information to industry and other users for better quality control, differentiation among subspecies, and processing variations (Suwonsichon, 2019). A well-defined and clarified lexicon would provide a comprehensive, but not redundant, vocabulary with related references that could be used for such things as quality control in industry and potentially could be associated with instrumental analysis to identify flavor compounds, such as alkyloids, terpenoids, flavonoids, fatty acids and other sorts of families. Also, the developed descriptive sensory lexicon can be associated with consumer acceptance or rejection (Lawless & Civille, 2013).

However, research is still lacking related to sensory terminology to describe the characteristics for Sichuan peppers. Therefore, the purpose of this research was to develop a lexicon and related standard references for characterizing Sichuan pepper. The lexicon should be provided in both English and in Chinese to make it widely available to potential users. An added objective is to characterize the sensory properties of 16 Sichuan pepper samples using the lexicon to determine if it discriminates among and can be used to group samples using statistical procedures.

Materials and Methods

Sichuan Pepper Samples

A total of 16 Sichuan Pepper samples were selected, provided and evaluated via aroma, and flavor profiles in the descriptive studies (Table 2.1). Six of the provided Sichuan Pepper samples were Green Sichuan Pepper, and 10 samples were Red Sichuan Pepper. All of the samples were Chinese in origin, but some were purchased in the United States.

Table 2.1 Test samples for descriptive analysis

Sample Name	Plant Location	Brands	Purchasing Location
Green 1	Jiangjin District, Chongqing City	Local Brand*	China
Green 2	Chongqing City	Local Brand	China
Green 3	Hanyuan County, Sichuan Province	Local Brand	China
Green 4	Chengdu City, Sichuan Province	Youjia	US
Green 5	Hanyuan County, Sichuan Province	Hanyuan Nature,. Co	US
Green 6	Yunnan Province	Local Brand	China
Red 1	Hancheng City, Shanxi Province	Local Brand	China
Red 2	Ya’an City, Sichuan Province	Local Brand	China
Red 3	Hancheng City, Shanxi Province	Local Brand	China
Red 4	Hanyuan County, Sichuan Province	Youjia	US
Red 5	Ya’an City, Sichuan Province	Local Brand	China
Red 6	Ya’an City, Sichuan Province	Local Brand	China

Red 7	Chongqing City	Local Brand	China
Red 8	Hunan Province	Local Brand	China
Red 9	Yunnan Province	Local Brand	China
Red 10	Chengdu City, Sichuan Province	Youjia	China

*Local brand refers to samples that typically are not labeled but sold by independent small retailers. They often are known simply by the name of the retailer.

Sample Preparation

The strong sensation of Sichuan pepper means that it typically is not eaten directly in the raw state. Because it often is used in soups and sauces the pepper samples for this study were evaluated as cooked samples in a liquid extract. All the Sichuan pepper samples (2 g) were soaked in 60 ml (1/4 cup) of hot water (80 C° ± 5 C°) in glass jars for 3 ½ min. For aroma attributes evaluation, the soaked Sichuan Pepper samples were removed from the water and the peppercorns were served in glass brandy snifters, which were covered with watch glass lids. For flavor attributes evaluation, the Sichuan Pepper water samples (60 ml) were served in glass jars on hot tiles covered in aluminum foil to maintain temperature. It should be noted that hot oil often is used with Sichuan Pepper during preparation, particularly with red pepper samples. This could impact the flavor compounds released into the mixture, but was not used in this research.

Panelists

Highly trained panelists (n=4) from the Center for Sensory Analysis and Consumer Behavior at Kansas State University participated in this project. Each panelist had over 120 h of general descriptive analysis training and over 1,500 hours of descriptive sensory experience, including testing white pepper, black pepper, and chili pepper. Such numbers of highly trained panelists have been shown to be able to discriminate among samples better than larger panels of less trained panelists (Chambers, Bowers & Dayton, 1981; Chambers & Smith, 1993; Chambers, Allison & Chambers, 2004). Similar panels have been used for other recent studies (Godoy et al., 2020; Adhikari et al., 2019; Di Donfrancesco et al., 2019; Chanadang & Chambers, 2019; Kumar & Chambers, 2019).

Panel Orientation

Three Red Sichuan pepper samples and three Green Sichuan pepper samples were used in the orientation step. Panelists discussed the samples and provided an initial lexicon including terms, definitions and references. During orientation they gave consensus scores to six samples (Chambers, 2018) to ensure they agreed on the terms of definitions and references. To find the best quantity for evaluation, the Sichuan Pepper samples (from 5g to 1 g) were placed in medium covered glass snifters and 60ml of the Sichuan Pepper water samples were served in glass jars covered by glass lids. After discussion, 2g of sample soaked in 60 ml hot water was chosen by the panelists as the best concentration for evaluation; that is, it provided a sufficient level of intensity, but did not overwhelm the palate.

Evaluation

All the samples were coded using three-digit random numbers. The panelists individually rated the intensity of aroma and flavor of each sample during three replications. A numeric scale of 0-15 with 0.5 increments, where 0 represented none and 15 represented extremely high, was applied to each attribute to provide a measure of intensity. The panelists were provided with purified (reverse osmosis, carbon filtered) water, mozzarella cheese, cucumber slices, unsalted crackers, and warm purified water for palate cleansing and panelists sniffed warm clean washcloths to clear the nose. Each session lasted 90 minutes during which panelists were served a total of five samples. Panelists could use up to 15 minutes of that time for evaluation, but typically spent less than 10 minutes during evaluation, leaving 3-8 minutes for palate cleansing.

Data Analysis

All the data was analyzed using XLSTAT 2019.2.1.58828 software (Addinsoft, MS Excel, NY, USA). Significant differences were determined using Analysis of Variance (ANOVA) at the 95% confidence interval ($\alpha = 0.05$).

Principal Components Analysis (PCA) was conducted to apply the covariance matrix in order to detect the relationship(s) among attributes and the Sichuan Pepper samples. Attributes were removed from the analysis that were scored two or fewer times during the evaluation by all panel members. This avoids having attributes that are scored only in a few cases impact the overall analysis. A PCA biplot presented the spatial arrangement for samples and attributes.

Cluster Analysis (CA) by Agglomerative hierarchical clustering (AHC) with Ward's method and visual inspection (Yenket & Chambers, 2017; Yenket et al., 2011) also was performed.

Results

Lexicon Development

Initially, 35 attributes were generated from panelists, included 17 aroma attributes and 18 flavor attributes in Table 2.2. Brothy aroma, smoke aroma, and sweet taste (flavor) attributes were scored by fewer than two panelists or scored infrequently in fewer than two sample and were removed from the final version after further discussion by panelists (Di Donfrancesco et al, 2012). “Brothy’ aroma was initially included to describe broth from cooked vegetables, but the panelists determined they were scoring this in “green” and other attributes. “Green” attributes have been described previously (Hongsoongnern & Chambers, 2008). “Smoke”’ aroma was included at first to describe a sharp, chemical feeling in the nose reminiscent of cedar wood or burnt notes. However, the panel determined during further evaluation that none of the samples had smoky burnt notes as described by Jaffe et al. (2017). Also, the lexicon had a citrus attribute that better described the sharp, chemical note in the nose, and a “woody” attribute to describe the cedar wood related attribute. Therefore, panelists decided to remove this attribute from final version. The attribute of ‘sweet’ taste was initially included in association with fruitiness and a “juicy” impression, but it was not used during actual testing. However, “sweet aromatics” associated with the smell of the samples was kept and may be related to the floral and fruity aroma noted in some products (Lotong et al., 2003).

Table 2.2 Initial Descriptive Lexicon for Sichuan peppers

Attributes			
Aroma		**Flavor**	
Sweet Aromatics (a)	Woody (a)	Green (f)	Pungent (f)
Peppery (a)	Fruity (a)	Citrus (f)	Umami (f)

Green (a)	Petroleum-like (a)	Minty (f)	Earthy (f)
Citrus (a)	Chive (a)	Fruity (f)	Piney (f)
Pungent (a)	Earthy (a)	Pepper (f)	Sweet (f)
Anise/Fennel (a)	Brown (a)	Petroleum-like (f)	Bitter (f)
Brothy (a)	Smoke (a)	Floral (f)	Astringent (f)
Perfumery/Floral (a)	Menthol (a)	Cardboard (f)	Tingle, Tongue (f)
Piney (a)		Green, Viney (f)	Numbing/Anesthetic (f)

(a) aroma attribute; (f) flavor attribute.

In the final lexicon (Table 2.3), a total of 32 sensory attributes were identified and detected by panelists: 15 aroma attributes and 17 flavor attributes. Lexicon development can have a number of terms to describe the products. For example, Lawless et al., (2012) found a total of 56 attributes to describe 35 spices, such as ginger, chili pepper, garlic, black pepper, etc. with each spice having five to 12 terms. Cabbage kimchi, another product with tingling and burning sensations required 18 attributes to describe (Chambers et al., 2012). More recently, Kumar and Chambers used 76 texture attributes to describe the broad range of textures, but those terms included ones such as "adhesiveness" that appeared in multiple contexts (hands, lips, teeth). Tran et al., (2019) used 28 flavor attributes to describe rye bread.

"Pungent", which in this study is not the same as heat/burn, was the most useful term to describe characteristics of Sichuan pepper (Yang., 2008; Riera et al., 2009). That term has been used to describe general sensations and combinations of sensations including burn, the chemical impression in the nose, the anesthesia symptoms on the tongue, and the special aroma of chili pepper. Such varying definitions for pungent have caused confusion in describing and identifying difference within peppers. For this research pungent was defined as a physically penetrating sensation in the nose. It did not relate to a specific aroma and flavor, such as sweet or citrus. “Numbing” (anesthesia) was applied to describe the loss sensation on the tongue and in the mouth, and “tingle” was related to the sensation of vibration on the tongue.

A bitter flavor note was detected among all samples and had high intensity scores. This flavor was not from caffeine in samples, but from the mixed alkylamides. β-sanshool was reported to provide bitter sensory characteristics an important component in Sichuan pepper (Sugai et al., 2005).

Table 2.3 Sichuan Pepper samples sensory attributes, definitions, references and intensities on a 15-point scale.

Attribute	Definitiion	Reference and Intensity
Aroma Attributes		
Anise/Fennel:	Aromatics typically associated with anise and fennel. Reminiscent of licorice candies or dark molasses	McCormick Anise Seed = 7.0 (aroma) Serve 1/2 tsp of seeds in a medium snifter, covered
Brown:	A rich full round aromatic impression always characterized as some degree of darkness generally associated with attributes such as toasted nutty, roasted, sweet.	Bush Pinto Beans (canned) = 6.0 (aroma) Drain beans and rinse with de-ionized water. Place one Tablespoon in a medium snifter at room temperature
Chive:	Green, slightly onion-like, sweet aromatic associated with fresh chives.	Freshly cut chives = 9.0 (aroma) Ten 1" pieces in a covered snifter
Citrus:	Slightly sharp, fruity aromatics associated with lemons and limes; may include peely notes.	Aura Cacia Lime Pure Essential Oil in cotton = 10.5 (aroma) 1 drop of Lime Oil in cotton in large snifters

Earthy:	Musty, somewhat sweet, full aromatics commonly associated with decaying vegetative matter and damp black oil.	Potting soil =10.0 (aroma) 1 Tbsp. of soil and 1/8 teaspoon water in medium jars
Fruity:	An aromatic blend of a variety of fruits, excluding citrus, cranberry, and concord grape, may include apples, pears, white grapes, etc.	Welch's White Grape Pear Juice= 9.0 (aroma) Dilute Welch's White Grape Juice with water of a 1:1 ratio, serve 2 Tbsps. in Median snifters
Green:	Aromatic characteristic of fresh plant-based material. Attributes may include leafy, viney, unripe, grassy, peapod	Fresh parsley water = 9.0 (aroma) Rinse and chop 25 g of fresh parsley. Add 300 ml of water. Let sit for 15 min. Filter. Serve 1 Tbsp. of the liquid part in a medium snifter.
Menthol:	The musty, sweet, pungent, and medicinal character of menthol.	0.015% Acros Menthol Solution = 4.0 (aroma) Place 2 drops of Menthol solution in cotton in a median snifter
Peppery:	A sharp biting, pungent aromatic associated with black pepper, white pepper, cumin, and chili pepper.	0.26g McCormick Black Pepper in 1 cup water = 5.0 (aroma) Add 0.26 g McCormick Black Pepper to 1 cup distilled water. Serve all of it in median size snifters.
Perfumery/Floral:	Floral aromatics with somewhat sweet, non-natural notes not generally associated with fresh fruit.	1 drop of IFF Geraniol in cotton =10.0 (aroma) Serve in large snifters
Petroleum-like:	A specific chemical aromatic associated with crude oil and its refined products that have heavy oil characteristics.	Vaseline Petroleum Jelly = 3.0 (aroma) Place a teaspoon of jelly in a medium covered snifter
Piney:	Aromatics reminiscent of resinous pine tree. Can be medicinal or disinfectant in character.	Pine-Sol Cleaner = 6.0 (aroma) Dilute Pine-Sol with water in a 1ml: 200 ml. Place mixture in large snifters, cover
Pungent:	A sharp physically penetrating	Heinz White Vinegar = 8.0

	sensation in the nose.	(aroma) Mix 1 part vinegar with 8 parts water. Serve in 1 oz. cup
Woody:	The sweet, brown, musty, flat, dark, dry aromatics associated with the bark of a tree.	ALDRICH Oil Cedar wood (Sigma) = 5.5 (aroma) One drop into cotton in large snifters
Sweet Aromatics:	Aromatics associated with the impression of sweet substance.	Lorna Doone =3.0 (aroma) Serve one crushed cookies in median size snifters
Flavor Attributes		
Cardboard:	The aromatics associated with cardboard or paper packaging. The intensity rating is only for the 'cardboard' character within the sample.	Mission Tortilla white flour = 2.5 (flavor) Serve in 3.25 oz. cups
Citrus:	Slightly sharp, fruity aromatics associated with lemons and limes; may include peely notes.	Fresh Lemon/Lime Juice, diluted = 5.0 (flavor) Mix equal parts of fresh lemon and lime juice. Dilute 1 part juice to 8 parts water. Serve in 1 oz. cups
Earthy:	'The slightly musty aromatics associated with raw potatoes and damp humus, slightly musty notes.	Fresh mushroom = 8.0 (flavor) 1 Tbsp. of Sliced Raw Button Mushroom in medium snifter, cover
Floral:	A light, smooth, mellow, soft, floral note.	Welch's White Grape Pear Juice= 5.0 (flavor) Dilute Welch's White Grape Juice with water of a 1:1 ratio
Fruity:	An aromatic blend of a variety of fruits, excluding citrus, cranberry, and concord grape, may include apples, pears, white grapes, etc.	Welch's White Grape Pear Juice= 5.0 (flavor) Dilute Welch's White Grape Juice with water of a 1:1 ratio
Green:	Aromatic characteristic of fresh plant-based material. Attributes may include leafy, viney, unripe, grassy, peapod	Fresh parsley water = 9.0 (flavor) Rinse and chop 25 g of fresh parsley. Add 300 ml of water. Let sit for 15 min. Filter. Serve 2 tsp in

		a 1 oz. cup
Green, Viney:	A green aromatic associated with green vegetables and newly cut vines and stems; characterized by increased bitter and musty/earthy character.	Campbell's Tomato Juice = 2.0 (flavor) Pour Campbell's Tomato Juice in 1 oz. cups, cover with lid
Minty:	The sweet, green, earthy, pungent, sharp, mentholic aromatics associated with mint oils.	Shea Moisture Peppermint oil in cotton ball = 9.0 (flavor) Place 1 drops of liquid in cotton in a median snifter
Pepper:	Aromatics associated with white and black pepper.	Black/White Pepper Mix= 9.0 (flavor) 0.12 grams McCormick Black Pepper and 0.12 grams McCormick White Pepper in 2 cups hot distilled water
Petroleum-like:	The aromatic associated with a petroleum product, described as clean, heavy, and oily.	Vaseline Petroleum Jelly = 3.0 (flavor) Place a teaspoon of jelly in a medium covered snifter
Piney:	Aromatics reminiscent of resinous pine tree. Can be medicinal or disinfectant in character.	Pine nuts = 5.0 (flavor) Serve pine nuts in 1 oz. cups
Pungent:	The sharp aromatics with a physically penetrating sensation in the nose.	Heinz Vinegar = 4.0 (flavor) Mix 10 mL vinegar to 200 mL water and serve individually in 1 oz. clear cups
Astringent:	The dry, puckering mouth feel associated with an alum solution in the mouth.	0.05 % Alum solution = 2.5 (flavor) 0.1%Alum solution=5.0 (flavor)
Bitter:	A fundamental taste factor of which caffeine is typical.	0.01% Caffeine solution = 2.0 (flavor) 0.035% Caffeine solution = 8.5 (flavor)
Numbing/Anesthetic:	The measurement associated with the perception of numbness on the tongue, roof of mouth, and/or	Chloraseptic = 9.0 (flavor) Serve half of candy in 3.25 oz.

	throat.	cups
Tingle, Tongue:	A feeling of an increased sensation on the tongue that may be due to chemical stimulation, intense carbonation, or other causes. Evaluate during first 3-5 seconds after sample is placed in the mouth.	7-Up = 8.5 (flavor) Serve before 15 min of panel
Umami:	Savory, salty, and somewhat flat, brothy aromatics/flavors associated with juices from cooked seafood, meat, and/or vegetables.	2 Button Mushroom Broth = 3.0 (flavor) Add 2 cups water and 2 medium-size button mushrooms (chopped) into a small sauce pan, bring to a boil and then boil for 5 min. Strain through a coffee filter and serve 1/4th cup liquid in medium snifter

The main characteristics of Sichuan Pepper samples are a Peppery attribute for both aroma and flavor profile, Green attribute for both aroma and flavor profile, Tingle Tongue attribute for mouthfeel and Numbing for mouthfeel. In total, 18 attributes were significantly different for the Green Sichuan Pepper and Red Sichuan Pepper samples: a) aroma: Peppery, Green, Pungent, Fennel Anise, Piney, Woody, Fruity, and Chive and b) flavor: attributes were Green, Peppery, Petroleum-like, Floral, Green Viney, Pungent, Piney, Bitter, Tingle Tongue, and Numbness.

Although some attributes were given for both aroma and flavor, that did not mean that they were necessarily different in both. For example, as shown Table 2.4 below, the attribute of fruity aroma was significantly different ($P \leqslant 0.05$) between Green and Red Sichuan Pepper samples, but there was no significant difference for the fruity flavor attribute between those same samples.

The definitions of the attribute are important because some reference products are the same in the test. For example, fruity aroma, fruity flavor, and floral flavor all use the same reference product (dilute Welch's White Grape Juice with water in a 1:1 ratio), but panelists still differentiated among the samples in different ways based on attribute definitions and characteristics of different samples. This supports that terms, definitions, and references are needed for the best differentiation among samples.

Comparison of Peppers

For most attributes, Green Sichuan Pepper samples were significantly higher ($P \leqslant 0.05$) than Red Sichuan Pepper samples (Table 2.4). That result shows that sensory attributes can be used to identify different subspecies of Sichuan Pepper, at least in terms of whether it is red or green.

Table 2.4 The ANOVA results for both subspecies of Sichuan Pepper samples.

	Samples	
Attributes	**Green**	**Red**
Sweet Aromaticss_aroma	2.0	1.9
Peppery_aroma	4.9 a	4.4 b
Green_aroma	6.5 a	5.9 b
Citrus_aroma	2.4	2.3
Pungent_aroma	3.1 a	2.7 b
Anise/Fennel_aroma	1.0 a	0.6 b
Perfumery/Floral_aroma	2.8	2.6
Piney_aroma	4.6 a	4.2 b
Woody_aroma	3.0 a	3.1 b

Fruity_aroma	2.2 a	1.7 b
Petroleum-like_aroma	3.5	3.4
Chive_aroma	1.0 a	0.7 b
Earthy_aroma	2.2	2.3
Brown_aroma	0.8	0.6
Menthol_aroma	1.8	1.9
Green_Flavor	7.3 a	6.4 b
Citrus_Flavor	2.2	2.1
Minty_Flavor	1.1	1.0
Fruity_Flavor	2.0	1.8
Pepper_Flavor	4.6 a	4.2 b
Petroleum-like_Flavor	4.1 a	3.3 b
Floral_Flavor	2.4 a	2.1 b
Cardboard_Flavor	2.3	2.3
Green Viney_Flavor	3.5 a	3.1 b
Pungent_Flavor	2.5 a	2.2 b
Umami_Flavor	2.6	2.5
Earthy_Flavor	2.6	2.5
Piney_Flavor	4.2 a	3.8 b
Bitter_Flavor	7.6 a	7.0 b
Astringent_Flavor	2.6	2.5
Tingle_Flavor	3.2 a	2.9 b
Numb_Flavor	3.4 a	3.1 b

a, b - Means with different letters within a row are significantly different at the 95% confidence interval.

Scores are based on a 15-point scale with 0.5 increments.

Within each subspecies, attributes were compared between individual samples for further identification purposes. As shown in the Table 2.5, most of the Green Sichuan Pepper samples were not significantly different ($P \leq 0.05$), except for tingling flavor. This was expected based on the lexicon development phase where panelists seem to see fewer differences among the green peppers. It may be that those green pepper samples are similar cultivars and ages of Sichuan Pepper even though they were different "brands". For Red Sichuan pepper samples, 14 attributes showed significant differences ($P \leq 0.05$): a) aroma: Sweet Aromatics, Peppery, Green, Pungent, Floral Perfumery, Piney, and Petroleum-like and b) flavor: Green, Floral, Pungent, Piney, Bitter, Tingle Tongue, and Numbness for the flavor profile. This may be because different cultivars of Red Sichuan Pepper were used to produce those samples. One piece of the supporting evidence is that Red Sichuan Pepper sample 5 and Red Sichuan Pepper sample 8 belong to two different categories coming from two distinct provinces in China: Sichuan and Hunan. The latitude and altitude of those two provinces are not the same, which means diverse regional climates, annual precipitation and solar irradiation are expected, which could lead to differentiated pepper samples. Cultivars also could be an important factor that would affect the sensory perception, but that is hard to know from this study since the specific cultivars are generally unknown for the samples.

Table 2.5 The ANOVA results of mean intensity scores for descriptive analysis of all 16 Sichuan Pepper samples.

	Attribute for Aroma														
Sample	Sweet Aromatics	Peppery	Green	Citrus	Pungent	Anise Fennel	Perfumery Floral	Piney	Woody	Fruity	Petroleum-like	Chive	Earthy	Brown	Menthol
Green 1	2.3 ab	5.2 a	6.4 abc	2.4	3.4 a	1.0	2.8 b	4.6	3.4 abc	2.2 abc	3.4 ab	1.4	2.2	0.9	1.7
Green 2	2.2 ab	4.8 ab	6.3 abc	2.4	3.1 a	1.2	3.0 ab	4.6	3.4 abc	2.0 abc	3.8 ab	0.8	2.3	0.8	1.9
Green 3	2.0 ab	4.8 ab	6.5 ab	2.3	3.0 a	1.2	2.8 b	4.6	3.8 a	2.3 ab	3.8 ab	0.8	2.3	1.1	1.9
Green 4	1.9 ab	5.1 a	6.1 abc	2.3	2.8 ab	0.8	2.5 b	4.3	3.7 ab	2.2 abc	3.5 ab	0.6	2.2	0.9	1.9
Green 5	2.3 ab	5.0 a	6.7 a	2.6	3.0 ab	0.9	2.7 b	4.7	3.5 abc	2.2 abc	3.2 ab	1.0	2.0	0.7	2.0
Green 6	1.4 ab	4.6 ab	6.8 a	2.4	3.3 a	0.9	3.0 ab	4.6	3.5 abc	2.5 a	3.2 ab	1.5	2.0	0.3	1.5
Red 1	1.6 ab	5.0 a	6.6 a	2.0	2.6 ab	0.9	2.5 b	4.4	3.3 abc	1.6 abc	4.2 a	0.7	2.4	1.2	1.7
Red 2	2.5 a	4.5 ab	7.0 a	2.5	2.9 ab	0.4	2.5 b	4.6	2.9 abc	1.7 abc	3.5 ab	0.7	2.4	0.8	2.1
Red 3	2.1 ab	4.5 ab	6.5 ab	2.3	3.3 a	0.5	2.6 b	4.4	3.3 abc	2.0 abc	3.7 ab	0.4	2.3	0.2	2.0
Red 4	2.7 a	3.6 b	4.0 d	2.1	3.0 ab	0.5	4.1 a	3.8	3.1 abc	2.2 abc	3.0 ab	0.6	2.0	0.5	1.7
Red 5	1.5 ab	4.5 ab	6.1 abc	2.2	2.5 ab	0.6	2.3 b	3.9	2.9 abc	1.1 b	3.1 ab	0.5	2.0	0.7	2.2
Red 6	1.9 ab	5.0 a	6.9 a	2.4	3.0 a	0.9	2.2 b	4.3	3.3 abc	1.7 abc	3.8 ab	0.5	2.5	0.7	2.0
Red 7	1.8 ab	4.1 ab	5.1 bcd	2.5	2.5 ab	0.3	2.5 b	4.6	3.3 abc	1.6 abc	3.6 ab	1.2	2.2	0.6	2.0
Red 8	1.9 ab	4.8 ab	6.3 abc	2.6	2.8 ab	0.6	2.8 b	4.6	3.0 abc	2.1 abc	3.7 ab	0.7	2.4	0.7	1.9
Red 9	2.3 ab	4.3 ab	5.0 cd	2.2	2.9 ab	0.4	2.5 b	3.8	2.7 c	1.5 abc	2.7 a	0.8	2.1	0.4	2.1
Red 10	1.0 b	4.0 ab	5.0 cd	2.1	1.8 b	0.7	2.0 b	3.7	2.8 bc	1.3 bc	3.0 ab	0.5	2.5	0.3	1.6

					Attributes for Flavor				
Samples	Green	Citrus	Minty	Fruity	Pepper	Petroleum like	Floral	Cardboard	Green Viney
Green 1	7.3 ab	2.4	0.9	2.2	5.0 a	4.0 abc	2.6 a	2.2	3.5
Green 2	7.7 a	2.2	1.6	2.3	4.3 ab	4.1 abc	2.3 ab	2.4	3.7
Green 3	7.0 ab	2.0	1.3	2.3	4.7 ab	4.3 ab	2.5 ab	2.3	3.3
Green 4	7.3 ab	2.1	0.6	1.6	5.0 a	4.6 a	2.1 ab	2.4	3.5
Green 5	7.0 abc	2.2	0.8	1.6	4.5 ab	3.5 abc	2.4 ab	2.2	3.4
Green 6	7.2 ab	2.4	1.3	1.9	4.2 ab	4.0 abc	2.2 ab	2.3	3.4
Red 1	7.0 ab	2.3	1.4	2.4	4.5 ab	3.8 abc	2.3 ab	2.4	3.2
Red 2	6.9 abc	2.1	0.9	1.8	4.8 ab	3.0 bc	2.6 a	2.3	3.0
Red 3	7.3 ab	2.1	1.5	1.9	4.5 ab	3.5 abc	1.7 ab	2.1	3.5
Red 4	5.2 e	1.9	1.2	1.9	4.2 ab	3.5 abc	2.4 ab	2.6	2.8
Red 5	6.8 abcd	1.9	1.4	1.7	4.6 ab	3.5 abc	2.2 ab	2.2	3.4
Red 6	6.6 abcd	2.2	0.8	1.8	4.3 ab	3.6 abc	2.0 ab	2.2	3.3
Red 7	6.2 bcde	2.1	1.0	1.7	3.8 ab	3.6 abc	2.1 ab	2.2	3.1
Red 8	6.3 bcde	2.0	0.6	1.6	3.8 ab	2.9 b	2.3 ab	2.5	3.1
Red 9	5.8 cde	2.0	0.6	1.6	3.9 ab	3.1 bc	2.2 ab	2.2	3.0
Red 10	5.5 de	2.0	0.6	1.5	3.5 b	3.1 bc	1.4 b	2.4	2.9

Attributes for Flavor								
Samples	Pungent	Umami	Earthy	Piney	Bitter	Astringent	Tingle	Numb
Green 1	2.5 ab	2.5	2.9	4.3 ab	7.6 a	2.7	3.4 ab	3.6 abc
Green 2	2.3 ab	2.7	2.6	3.9 ab	7.6 a	2.5	2.5 b	2.8 bcd
Green 3	2.3 ab	2.6	2.4	4.4 ab	7.4 ab	2.6	3.4 ab	3.7 abc
Green 4	2.6 a	2.7	2.7	4.6 a	7.6 a	2.5	3.3 ab	3.9 ab
Green 5	2.7 a	2.5	2.5	4.3 ab	7.6 ab	2.6	3.2 ab	3.2 abcd
Green 6	2.4 ab	2.3	2.7	4.0 ab	7.5 ab	3.0	3.4 ab	3.5 abc
Red 1	2.2 ab	2.8	2.9	3.9 ab	7.0 ab	2.4	2.8 b	3.0 bcd
Red 2	2.2 ab	2.5	2.7	3.8 ab	6.8 ab	2.4	3.0 ab	3.0 bcd
Red 3	2.6 a	2.5	2.5	4.0 ab	7.2 ab	2.5	3.2 ab	2.9 bcd
Red 4	2.1 ab	2.6	2.8	3.9 ab	7.4 ab	2.8	3.3 ab	3.7 abc
Red 5	2.6 a	2.6	2.5	4.5 a	7.3 ab	2.6	4.0 a	4.3 a
Red 6	2.1 ab	2.4	2.3	3.5 ab	7.3 ab	2.5	2.8 b	2.6 cd
Red 7	2.0 ab	2.6	2.5	4.2 ab	7.3 ab	2.5	3.0 ab	3.3 abcd
Red 8	2.3 ab	2.4	2.5	3.6 ab	6.8 ab	2.3	2.3 b	2.3 d
Red 9	2.0 ab	2.6	2.4	3.7 ab	6.7 ab	2.5	2.5 b	2.8 bcd
Red 10	1.5 b	2.2	2.5	3.3 b	6.5 b	2.2	2.3 b	2.9 bcd

a, b, c, d - Means with same letter within a row are not significantly different from each other at the 95% confidence interval.

Scores are based on a 15-point scale with 0.5 increments.

In this study, the PCA was conducted to illustrate the relationship between samples and their sensory attributes. As shown in the biplot (Figure 2.1), a total of 62.6% of the variation was explained in the first two principal components of the PCA. Among the 16 Sichuan Pepper samples, the six Green Sichuan Pepper samples are more positively related than the red pepper samples to ‘Peppery flavor’, ‘Pungent flavor’, ’Pungent aroma’, ‘Woody flavor’, ‘Fruity flavor’, ‘Green Viney flavor’, ‘Anise Fennel aroma’, ‘Brown flavor’, and ‘Piney aroma’. One issue with using such biplots however, is that the analysis examines overall relationships, not single attributes (Yenket et al., 2011). For example, the scores for ‘Numbness’ and ‘Tingle’ of the Green Sichuan Pepper samples generally were significantly higher than those scores for Red Sichuan Pepper. However, the single highest intensity scores for ‘Numbness’ and ‘Tingle’ were from the Red Sichuan Pepper sample.

Generally, the Green Sichuan Pepper samples are more closely located with each other in PCA Biplot than Red Sichuan Pepper samples suggesting more similarity among the green samples than the red ones. Red Sichuan Pepper sample 1 and Red Sichuan Pepper sample 3 are located closely to each other, which were from same city (Hancheng City, Shanxi Province). Red Sichuan Pepper sample 2 and Red Sichuan Pepper sample 6 are located closely to each other as well and those two samples also are from same city (Yaan City, Sichuan Province). Although Red Sichuan Pepper sample 5 is also from that city (Yaan City, Sichuan Province), it is not located close to Red 2 or Red 6, suggesting it was a different cultivar, grown under different conditions, or is a different age than those samples. The rest of Sichuan Pepper samples were different cultivars from different cities and provinces and are in different locations in the Biplot. Considering that the Green Sichuan Pepper samples were from several different provinces as well, the conclusion that Red Sichuan Pepper samples might be affected by cultivar and region more than Green Sichuan Pepper samples appears possible.

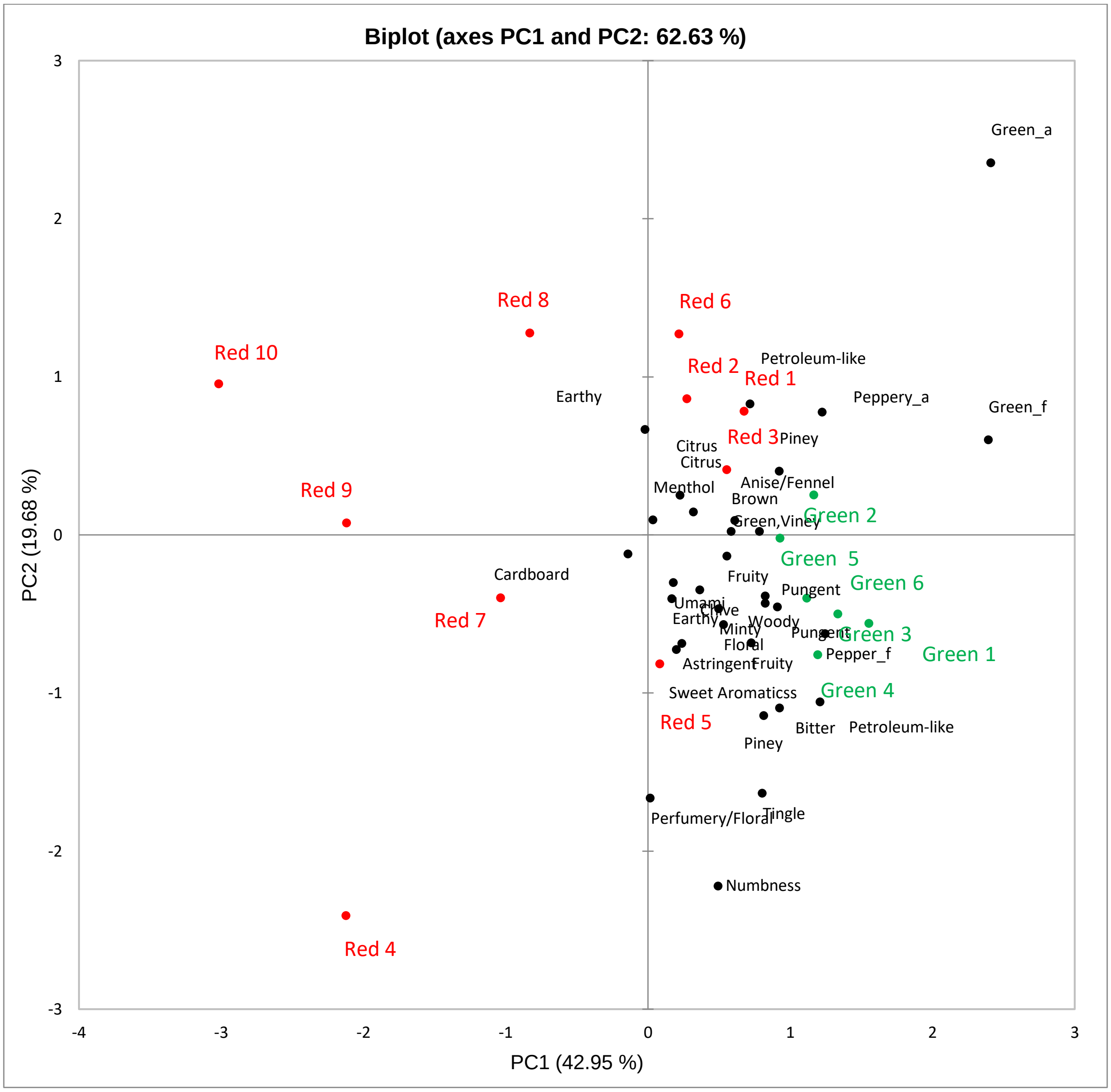

Figure 2.1 Sichuan Pepper samples profiles by using Principal Components Analysis – Biplot of PC1 and PC2.

The Agglomerative hierarchical clustering (AHC) analysis helps to understand the clusters among these 16 Sichuan Pepper samples and the results are shown as in Figure 2.2 Typically, Sichuan Peppers were divided into Red Sichuan Pepper and Green Sichuan Pepper groups in their sensory properties. The Red Sichuan Peppers are distinguished by their milder aroma characteristics, in general, and for some samples, their higher bitterness and numbing characteristics. This clustering into groupings of red and green again suggests that the 32 attributes determined in this study are able to distinguish different subspecies, Green and Red, in Sichuan Peppers. There is one exception; Red Sichuan Pepper sample 1 was assigned to Green Sichuan Pepper group in AHC. This is surprising given the PCA shows the key characteristics attributes for this sample are much closer to the Red Sichuan Pepper group, more specifically, similar to Red Sichuan Pepper sample 3. This shows the value of using different analyses for such research. It may be that the Red Sichuan Pepper sample has some sensory properties similar to both subspecies.

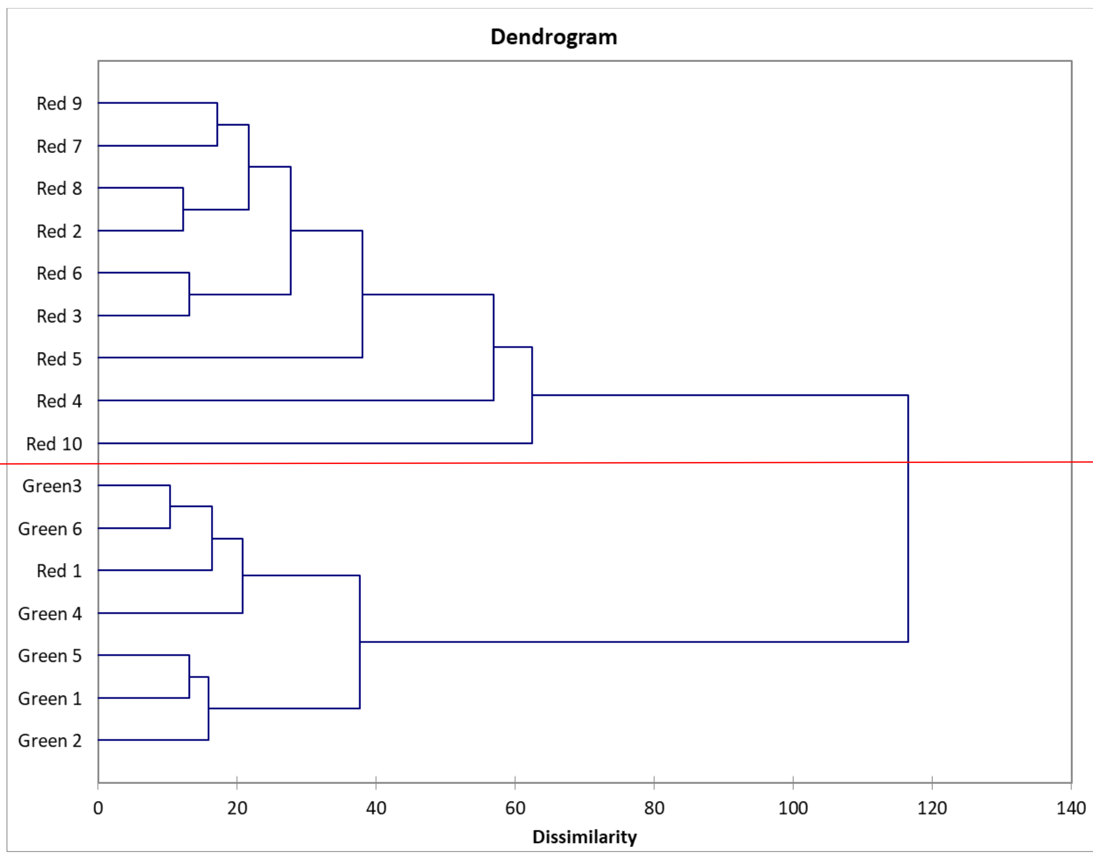

Figure 2.2 Agglomerative hierarchical clustering (AHC) of sensory characters for Sichuan Pepper samples

Limitations

A few limitations in this study should be addressed in future studies. First of all, Sichuan Pepper samples were evaluated after soaking in hot water. Thus, some chemicals might be destructed before sensory evaluation. The characteristic chemical components of Sichuan Pepper are alkaloids and terpenoids, which provided the tingle, numb and floral sensation. These chemicals are difficult to dissolve in water although such preparations are typical for use. In traditional dishes, the food material could absorb some of the extract or result in a break-down of the chemical structure. It also is possible that in water, the high temperature might result in evaporation of some volatile chemicals, reducing the intensity of perception. Alternative methods for soaking or cooking the peppers, such as in oil or an oil and water mixture might be considered. However, such extracts are hard to taste and the oil could be easily hydrolyzed or oxidized, resulting in changes to the sensory profile.

In traditional dishes, such as “Hot-Pot”, the Sichuan Pepper is mixed with salt, chili pepper, cayenne, and other seasonings which could enhance, decrease, or otherwise modify some sensory properties of Sichuan Pepper.

A final consideration is that although the 16 Sichuan Pepper samples used in this study were carefully and widely chosen to represent many regions of China, they generally are of unknown cultivar and age and reasonably cannot cover the full ranges and subspecies in all Sichuan Peppers available in China and other areas. Chambers et al. (2016) suggested that development of such lexicons must be considered as preliminary and changes can be made as additional samples either through breeding, processing, or application are included.

Conclusions

In this study, 16 Sichuan pepper samples were evaluated to develop and validate a lexicon to help standardize future evaluation. As the result, a total of 32 sensory attributes were developed, defined, and referenced for descriptive sensory analysis. The main characteristics of Sichuan Pepper samples are Peppery and Green attributes for both aroma and flavor and Tongue Tingle and Numbness for mouthfeel. A number of attributes showed significant differences among red and green subspecies of peppers. Future studies should include more different samples of Sichuan pepper to uncover further attributes.

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Chapter 3 - Instrumental Analysis on Different Subspecies of Sichuan Pepper

Abstract

In order to further understand Sichuan Pepper volatile compounds characteristics, an instrumental analysis on different subspecies of Sichuan Pepper samples is conducted. The objective of this study were to a) understand volatile compounds of Sichuan peppers, and b) associate the volatiles with descriptive sensory analysis information. Gas chromatography-mass spectrometry (GC-MS) associated with a modified headspace solid-phase microextraction and descriptive sensory analysis was applied to total of 16 Sichuan Pepper samples differentiated by age and color, such as green Sichuan pepper and red Sichuan pepper. Total of 99 volatiles were tentatively detected among 16 samples. Total 46 terpenoid compounds were detected which was the most abundant group of volatiles. Partial least squares regression was used to associate the sensory characteristics to volatile compounds. Possible relationships between chemicals and sensory attributes were uncovered, in which the most prominent associations included β-Copaene related to Anise/Fennel aroma and Fruity aroma, and Petroleum-Like flavor, α-Phellandrene related to Earthy aroma, cis-Carveol and 2-Phenylethyl acetate related to Perfumery/Floral aroma, Sabinene hydrate related to Woody aroma and Petroleum-Like flavor, (+)-β-Thujone related to Anise/Fennel aroma, Fruity aroma, Woody aroma, Green flavor, Petroleum-Like flavor, and Green Viney flavor, β-Pinene related to Peppery aroma and Citrus flavor, δ-Limonene related to Menthol aroma, Linalyl acetate related to Pepper flavor, and Anise Fennel aroma. Results suggested that Sichuan pepper volatiles change over maturation.

Introduction

Sichuan Pepper, also known as *HuaJiao* (Chinese) which belongs to *Zanthoxylum* genus (Rutaceae), is a typical spice in Chinese cuisines and consists of 42 species and 14 varieties in China, more than 246 species worldwide. Its unique sensory property was described as 'numbing' or 'pungent' (Yasuda et al., 1982; Yang., 2008) distinguished from other peppers like chili, black or white peppers. To understand the main components of Sichuan Pepper, over 140 compounds have been isolated and identified from different Sichuan Pepper cultivars, including alkaloids, terpenoids, free fatty acids and flavonoids (Yasuda et al., 1982; Mizutani et al., 1988; Xiong et al., 1997; Yang, 2008; Xia et al., 2011; Yang et al., 2013). The unsaturated alkylamids were extracted and isolated from various *Zanthoxylum* genus which reported to provide the 'numbing' or 'pungent' taste, such as hydroxy-α-sanshool, hydroxy-β-sanshool, hydroxy-γ-sanshool, hydroxy-ε-sanshool, γ-sanshool, hydroxy-γ-isosanshool, dehydro-γ-sanshool (Yasuda et al., 1982; Xiong et al., 1997). Further studies reported that hydroxy-α-sanshool would be the primary substance to provide the 'numbing' sensation due to its *cis* and *trans* double bonds, but not all *trans* double bonds such as hydroxy-β-sanshool were tasteless (Mizutani et al., 1988).

The extracted oil from Sichuan Pepper has strong flavor characteristics. By gas chromatography coupled with mass spectrometry, terpenoids were found to be main compounds in these extracted oil by their high ratio. Linalyl acetate, linalool, limonene, and sabinene were found in many Sichuan Pepper cultivars and were major components (Yang., 2008; Liu et al., 2017). But the chemical components of terpenoids are different in different studies which might be affected by variety, origin and climate, extraction methods and other factors (Chen et al., 2015; Wang et al., 2010). Except terpenoids, the extracted oil contains many small molecular substances such as olefins, ketones, alcohols, esters, and epoxies (Chyau et al., 1996; Zhang et al., 2017)

Given the complexity of chemical compounds, some studies have tried to detect the association between chemical components and sensory properties of Sichuan Pepper except numbing sensation. Frerot and others (2015) reported the umami amide (E)-3-(3,4-Dimethoxyphenyl)-N-(4-methoxyphenethyl)acrylamide was found in in the roots and stems of *Zanthoxylum piperitum.* Ding and others (2017) showed the bitter taste would relate to the alcohol and ketone families of chemical components. However, no research was found on the relationship between full of sensory profiles and chemical components. Therefore, the purpose of this study was to associate the relationship of sensory profiles and chemical compounds. Also, we tried to understand how these chemical components separated and grouped among Sichuan pepper samples.

Methods

Sichuan Pepper Samples

Total of 16 samples are applied in instrumental analysis, and the samples are as same as Sichuan pepper samples described in Chapter 2, Sichuan Pepper Samples section.

Extraction Methods of Volatile Chemicals

The extraction method for studying the aroma profile in the samples was headspace solid phase micro extraction (HS-SPME), similar to what was done by Koppel et al. (2013). The samples were ground in a grinder for 10 sec. About 0.1 g sample was weighed in a 10 mL screw-cap vial (Supelco Analytical, Bellefonte, PA, USA) equipped with a polytetrafluoroethylene/silicone septum (Supelco Analytical, Bellefonte, PA, USA). Exactly 0.5 mL distilled water was added to the ground sample in the vial, and 10miL 100 ppm 1,3-dichlorobenzene dissolved in methanol (Sigma Aldrich, St. Louis, MO, USA) as the internal standard. The sample was incubated at 50 °C for 1 min, and then a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco Analytical, Bellefonte, PA, USA) was exposed to the sample headspace for 5 min at 50 °C. After sampling, the analytes were desorbed from the SPME fiber coating to the GC injection port at 150°C for 3 min in splitless mode.

Chromatographic Analysis

The isolation, tentative identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (Shimadzu GC-2010 Plus), coupled with a Shimadzu mass spectrometer (MS) detector (GCMS-QP2020). The GC-MS system was equipped with an SH-Rxi-5Sil MS Crossbond column (Shimadzu, Tokio, Japan; 30 m × 0.25 mm × 0.25 μm film thickness). The column was heated from 40 °C to 200 °C. The Ion Source was set at 200°C and the mass spectrometer scanned for masses between 35 and 350 m/z. Volatile compounds were identified using NIST library version 14. All analyses were run in 3 replicates. The semi-quantification of volatile compounds was manifested by the ratio of peak area, which was calculated by the GC peak area divided by the peak area of internal standard.

Descriptive analysis method for sensory characteristics

In the first part of this study to detect the differences of sensory characteristics for 16 Sichuan Pepper samples, descriptive sensory analysis method was applied. Total of 5 highly trained panelists from Center for Sensory Analysis and Consumer Behavior, Kansas State University (Manhattan, KS, USA) participated in this study. All panelists had been trained in general descriptive sensory analysis panel 120 hours and over 1000 hours experience in descriptive sensory analysis panels. The training included identification for sensory characteristics, development for terminology and definition, and quantifying intensity for sensory characteristics. All Sichuan Pepper samples (5g) were boiled in water (60ml) at temperature 176° ± 9° for 3 ½ min. Boiled Sichuan Pepper pericarps samples (5g) were served to each panelist in medium size snifters covered with a watch glass for aroma characteristics. The Sichuan Pepper water samples (60ml) were severed to each panelist in mason jars covered with a watch glass for flavor characteristics separately.

Total of 15 aroma sensory characteristics and 17 flavor sensory characteristics were detected. The aroma characteristics terms included: sweet aromatics, peppery, green, citrus, pungent, anise fennel, perfumery floral, piney, woody, fruity, petroleum-like, chive, earthy, brown, and menthol. The flavor sensory characteristics terms included: green, citrus, minty, fruity, pepper, petroleum-like, floral, cardboard, green viney, pungent, umami, earthy, piney, bitter, astringent, tingle, and numb. All these sensory characteristics terms were verified and scored by panelists individually on a 15-point scale from 0 to 15 with 0.5 increments with references provided for different scale points.

Data Analysis:

Statistical software was performed by XLSTAT, version 2019.2.1.58828 (Addinsoft, MS Excel, NY, USA). Agglomerative hierarchical clustering (AHC) help to cluster samples by chromatographic analysis data. Partial least square regression (PLSR) was performed to identify associations between chromatographic analysis data and descriptive sensory analysis data. The (Koppel et al., 2013; Lee et al., 2011; Di Donfrancesco et al., 2017) also applied PLSR to determine correlations between volatile compounds and sensory characteristics of food. Specifically, in PLSR analysis, the volatile compounds whose content less than 0.5 µg/kg were excluded in order to keep the ones that have more prominent values only.

The volatile compounds whose content less than 0.5 µg/kg were excluded in order to keep the ones that have more prominent values only. Therefore, 40 out of 99 volatile compounds were kept in PLSR analysis, which are listed in the Table 3.1 as below.

Table 3.1 Volatile Compounds in PLSR Analysis

	Volatile Compounds		
Myrtenal	Pinene	β-Copaene	(1R,4R,5S)-1-Isopropyl-4-methoxy-4 methylbicyclo[3.1.0]hexane
α-Phellandrene	α-Terpinyl acetate	β-Myrcene	β-Phellandrene
γ -Terpinene	(Z)-β-Ocimene	α-Terpinene	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene
Neryl acetate	2-p-Menthen-1-ol	cis-Carveol	6-(Isopropyl)-3-methylcyclohex-2-en-1-yl acetate
Piperitone	4-Isopropyl-2-cyclohexenone	(-)-Terpinen-4-ol	4-Terpinenyl acetate
2-Phenylethyl acetate	Methyl acetate	Octyl acetate	α-Thujene
Sabinene hydrate	(+)-β-Thujone	β-Pinene	Caryophyllene
δ-Limonene	Eucalyptol	Germacrene D	Hexanal
L-α-Terpineol	Linalool	Linalyl acetate	Linalyl isobutyrate
γ-Cadinene	β-Cadinene	β-Selinene	o-Cymene

All compounds names are referred to PubChem. (https://pubchem.ncbi.nlm.nih.gov/)

The results from this study and previous studies showed difficulties to identify and uncover the relationship between sensory characteristics and chemical compounds for single cultivar of Sichuan Pepper. Even sample from same cultivar, different extraction methods, ages, and growth conditions would affect the result of volatile compounds (Wang et al., 2010). Therefore, we grouped Sichuan Pepper samples by their ages instead of single cultivar to detect how their volatiles affect sensory characteristics.

Results and Discussion

Volatile compounds

Total of 99 volatile compounds were detected among 16 Sichuan Pepper samples (Table 3.2, Table 3.3). These volatile compounds included 46 terpenoid compounds, 15 ester compounds, 10 alcohol compounds, 8 alkene compounds, 5 aldehyde compounds, 4 alkane compounds, 3 ketone compounds, and 1 other group compounds (included xanthoxylin, pinene, humulene epoxide II, β-dihydroagarofuran, acetic acid, and valencene).

Terpenoids were the most abundant components in Sichuan Peppers considering the high rationale (Table 3.1). In this study, terpenoids concentration varied from 26.22 μg/kg to 163.85 μg/kg varied in Sichuan Pepper samples, and the percentage of terpenoids in each sample varied from 80% to 90%. The main compounds in terpenoids were α-Terpinyl acetate (0.04 to 0.84 μg/kg in Green Sichuan Pepper, 0.60 to 5.90 μg/kg in Red Sichuan Pepper), beta-Myrcene (2.38 to 7.65 μg/kg in Green Sichuan Pepper, and 3.8 to 11.72 μg/kg in Red Sichuan Pepper), γ-Terpinene (0.30 to 2.05 μg/kg in Green Sichuan Pepper, 0.09 to 5.12 μg/kg in Red Sichuan Pepper), Terpinen-4-ol (1.93 to 9.68 μg/kg in Green Sichuan Pepper and 0 to 11.91 μg/kg in Red Sichuan Pepper), Sabinene hydrate (3.54 to 6.32 μg/kg in Green Sichuan Pepper and 0 to 3.63 μg/kg in Red Sichuan Pepper), Limonene (0 to 12.67 μg/kg in Green Sichuan Pepper and 0 to 25.56 in Red Sichuan Pepper), Eucalyptol (only in Red Sichuan Pepper from 6.24 to 22.29 μg/kg), Linalool (only in Red Sichuan Pepper from 13.57 to 33.43 μg/kg), Linalyl acetate (3.89 to 11.25 μg/kg in Green Sichuan Pepper, and 0 to 10.26 μg/kg in Red Sichuan Pepper), Linalyl isobutyrate (only in Red Sichuan Pepper from 0 to 80.99 μg/kg). To compare these main compounds, except Sabinene hydrate and Linalyl acetate, Red Sichuan Pepper samples had higher content than Green Sichuan Pepper samples had. But to compare these main compounds by rationale of each sample, Green Sichuan Pepper had higher rationale than Red Sichuan Pepper. Except above terpenoids compounds, we also detected many monoterpenes and sesquiterpenoids. The terpenoids have been shown to play an important role in aroma contribution. But the contents of terpenoids compounds found in this study has little different to compare with previous studies. For example, linalool believed to be the major aroma component for both Green and Red Sichuan Pepper (Yang., 2008). However, in this study, linalool is the only major aroma component for Red Sichuan Pepper, and was not detected in Green Sichuan Peppers. It could be explained by different growth conditions, extraction methods, genetic characteristics, ages and other factors (Wang et al., 2010; Li et al., 2014).

Other studies also detected terpenoids as a main volatile compounds in Sichuan Peppers, and α-Limonene was the most abundant compound in Sichuan Pepper (Liu et al., 2017). The terpenoids of linalool, α-terpineol, myrcene, 1,8-cineole, Linalyl acetate, limonene, and geraniol were identified as important factors to affect aroma between Green and Red Sichuan Pepper. The aroma of Red and Green Sichuan Pepper was described as floral, pungent, cooling, green, and refreshing (Yang ., 2008). Jiang & Kubota (2004) reported that the main chemicals of the essential oil extracted from Sichuan Pepper, genus of *Zanthoxlum piperitum* from Japan, were limonene (29.54%), β-phellandrene (17.79%), geranyl acetate (17.71%), geraniol (8.9%), and citronellal (7.26%). For the essential oil extracted from leaves and branches of Sichuan Pepper, eucalyptol, β-terpinene, piperitone, safrole, methyl eugenol, and β-Caryophyllene were major component and existed in several genus of Sichuan Pepper Zhang et al., (2017). Wijaya et al., (2002) reported that wild genus of Sichuan pepper, *Zanthoxylum acanthopodium* in northern Sumatera, Indonesia, has an exotic citrus-like aroma. The major essential oil components are geranyl acetate (32.04%) and limonene (15.8%).

Esters were the second largest group of volatiles found, and the concentration was not high. The average concentration of total esters for Red Sichuan pepper was 2.31 µg/kg, and for Green Sichuan Pepper was 0.65 µg/kg. Specifically, 2-phenylethyl acetate (0 to 0.24 µg/kg in Green Sichuan pepper, 0 to 0.53 µg/kg in Red Sichuan Pepper) and isobutyl acetate (0 to 0.03 µg/kg in Green Sichuan Pepper, 0.01 to 0.07 µg/kg in Red Sichuan Pepper) were detected. Yang ., (2008) also reported 2-Phenylethyl acetate (0.065% in Green Sichuan Pepper, 0.089% in Red Sichuan Pepper), and isobutyl acetate (0.028% in Green Sichuan Pepper, 0.266% in Red Sichuan Pepper).

Eight alkenes were detected among samples. The average concentration of total alkenes for Red Sichuan pepper is 1.03 µg/kg, and for Green Sichuan Pepper was 3.22 µg/kg.

Four Ketones were detected among samples. The average concentration of total ketones for Red Sichuan pepper was 0.83 µg/kg, and for Green Sichuan Pepper was 1.93 µg/kg.

Ten Alcohols were detected among samples, but only in a small amount. The average concentration of total alcohols for Red Sichuan pepper was 0.25 µg/kg, and for Green Sichuan Pepper was 0.13.

Aldehydes, and alkanes only took a small amount among samples. The average concentration of total aldehydes for Red Sichuan pepper was 0.15 µg/kg, and for Green Sichuan Pepper was 0.10 µg/kg. The average concentration of total alkanes for Red Sichuan pepper was 0.3 µg/kg, and for Green Sichuan Pepper was 0.52 µg/kg.

Table 3.2 Content (μg/kg) of volatile compounds in the 6 different Green Sichuan Pepper samples, SD: standard deviation, N.D: not detected. RI – Retention Time

Sample			Green 1	Green 2	Green 3	Green 4	Green 5	Green 6
Code	**Compounds**	**RI**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**
			Terpenoids					
C63	β-Elemene	18.81	0.29±0.06	N.D	0.06±0.01	0.05±0.07	N.D	N.D
C33	*cis-Carveol	14.42	0.06±0.01	0.21±0.03	0.05±0	0.3±0.03	0.41±0.06	0.08±0
C40	*(-)-Terpinen-4-ol	13.53	2.47±0.65	1.93±0.15	2.43±0.39	9.68±1.13	3.65±0.77	2.21±0.18
C35	(+)-Isopiperitenone	15.75	0.03±0.01	N.D	N.D	0.07±0.02	0.06±0.03	0.04±0
C52	*(+)-β-Thujone	12	1.93±0.03	2.73±0.23	1.78±0.05	2.36±0.27	2.82±0.24	1.71±0.1
C1	*Myrtenal	13.93	N.D	0.75±0.04	N.D	1.12±0.17	0.77±0.08	N.D
C2	*Pinene	7.74	2.57±1.46	0.49±0.12	1.07±0.25	0.66±0.39	0.34±0.32	1.08±0.38
C20	*(Z)-β-Ocimene	10.02	1.93±0.79	0.35±0.11	0.61±0.14	0.52±0.24	0.27±0.1	0.62±0.16
C39	2-Acetoxy-1,8-cineole	17.45	N.D	N.D	N.D	N.D	N.D	N.D
C32	*2-p-Menthen-1-ol	12.5	N.D	0.25±0.02	0.25±0.07	0.55±0.02	0.34±0.03	0.27±0.01
C37	*4-Isopropyl-2-cyclohexenone	13.68	0.14±0.06	0.53±0.07	0.16±0.02	0.56±0.02	0.53±0.06	0.10±0.01
C42	*4-Terpinenyl acetate	17.3	0.23±0.16	0.07±0.01	N.D	N.D	N.D	0.15±0.08
C54	Bornyl acetate	16.12	0.21±0.05	N.D	N.D	N.D	N.D	N.D
C58	Camphene	8.09	0.04±0.03	0.01±0	0.01±0	0.02±0.01	0.01±0.01	0.02±0.01
C59	Carveol	14.79	N.D	0.13±0.02	N.D	0.13±0.01	0.18±0.04	0.08±0
C97	Carvyl acetate	17.91	N.D	N.D	N.D	N.D	N.D	N.D

C60	*Caryophyllene	19.68	3.51±0.33	2.06±0.26	3.19±0.56	1.62±0.68	1.4±0.82	2.66±0.49
C61	Caryophyllene oxide	23.54	0.03±0.01	0.19±0.04	0.04±0.01	0.19±0.02	0.13±0.11	0.04±0.03
C62	Citronellal	12.61	0.35±0.02	0.22±0	0.18±0.16	N.D	N.D	0.21±0.01
C64	δ-Terpineol	13.2	N.D	N.D	N.D	N.D	N.D	N.D
C16	δ-Terpineol acetate	16.81	0.05±0.01	N.D	0.05±0.01	0.03±0	N.D	0.04±0.01
C66	*δ-Limonene	9.79	N.D	N.D	12.68±2.77	N.D	N.D	N.D
C68	*Eucalyptol	9.94	N.D	N.D	N.D	N.D	N.D	N.D
C82	*γ-Cadinene	21.86	0.33±0.04	0.08±0.01	0.33±0.04	0.25±0.06	0.07±0.03	0.18±0.02
C69	Geranyl acetate	18.31	N.D	N.D	N.D	N.D	N.D	N.D
C23	Germacrene B	23	0.05±0.02	N.D	0.05±0.01	N.D	N.D	0.03±0
C70	*Germacrene D	21.14	N.D	N.D	N.D	N.D	N.D	N.D
C77	*Linalool	11.39	N.D	N.D	N.D	N.D	N.D	N.D
C78	*Linalyl acetate	15.13	11.25±0.45	4.19±3.71	9.62±1.11	5.03±0.67	3.90±3.39	7.37±6.4
C79	*Linalyl isobutyrate	15.32	N.D	N.D	N.D	N.D	N.D	N.D
C76	*L-α-Terpineol	13.85	1.57±0.32	N.D	1.81±0.14	2.10±0.06	1.34±0.16	1.30±0.08
C53	*β-Pinene	8.673	2.09±1.13	0.56±0.17	0.77±0.12	0.67±0.37	0.47±0.48	0.88±0.29
C31	*Neryl acetate	17.82	0.07±0.02	0.01±0	0.07±0.01	0.02±0	0.02±0.01	0.06±0.01
C90	*o-Cymene	9.64	N.D	1.00±0.27	0.49±0.06	3.33±1.2	1.49±0.62	0.46±0.04
C50	*Sabinene hydrate	11.79	3.55±2.78	5.11±0.32	4.67±0.19	6.33±0.47	5.57±0.34	4.48±0.06
C9	α-Cadinol	25.1	N.D	N.D	N.D	N.D	N.D	N.D
C10	α-Calacorene	22.51	N.D	N.D	N.D	N.D	N.D	N.D

C11	α-Cubebene	17.76	0.09±0.01	0.03±0.01	0.09±0.01	0.06±0.01	N.D	0.07±0
C12	*α-Phellandrene	9.24	N.D	N.D	N.D	N.D	N.D	N.D
C21	*α-Terpinene	9.46	1.21±0.47	0.56±0.12	0.83±0.1	0.75±0.29	0.60±0.22	0.90±0.1
C13	*α-Terpinyl acetate	17.65	0.79±0.07	0.05±0.03	0.84±0.15	0.17±0.01	0.04±0.05	0.80±0.1
C49	*α-Thujene	7.561	2.09±1.42	1.03±0.29	1.18±0.24	1.15±0.6	0.99±0.72	1.53±0.46
C86	*β-Cadinene	21.94	0.30±0.07	0.08±0.01	0.30±0.03	N.D	0.07±0.04	0.20±0.01
C14	*β-Myrcene	8.759	7.66±2.88	2.99±0.97	2.86±0.8	3.09±1.61	2.39±1.78	3.44±1.01
C15	*β-Phellandrene	9.82	N.D	N.D	3.24±0.41	N.D	N.D	N.D
C18	*γ - Terpinene	10.4	1.51±1.5	1.13±0.23	0.31±0.22	2.05±0.75	1.40±0.56	0.70±1.12
C19	τ-Cadinol	24.79	0.02±0.01	N.D	0.02±0.01	0.02±0	N.D	N.D
	Total Terpinoids		43.8	26.25	48.89	42.22	28.92	30.53
			Esters					
C8	(E)-2-Methyl-2-butenyl isobutyrate	5.34	N.D	0.01±0	N.D	N.D	N.D	0.01±0
C34	*6-(Isopropyl)-3-methylcyclohex-2-en-1-yl acetate	16.38	N.D	N.D	N.D	N.D	N.D	N.D
C25	Isoamyl acetate	6.54	0.03±0.02	N.D	0.02±0	N.D	N.D	N.D
C75	Isobutyl acetate	5.09	N.D	N.D	N.D	N.D	N.D	N.D
C56	Isobutyl butyrate	8	N.D	N.D	N.D	N.D	N.D	N.D
C45	*Methyl acetate	3.11	0.25±0.05	0.41±0.04	0.34±0.13	0.45±0.03	0.36±0.19	0.39±0.06
C57	Methyl butyrate	4.48	N.D	N.D	0.01±0.01	N.D	N.D	0.01±0.01
C98	Methyl geranate	16.91	N.D	N.D	0.02±0	N.D	N.D	0.01±0

C74	Methyl hexanoate	7.41	N.D	N.D	N.D	0.01±0	N.D	N.D
C89	Methyl octanoate	11.84	N.D	N.D	N.D	N.D	N.D	N.D
C80	Methyl valerate	5.77	N.D	N.D	N.D	N.D	N.D	N.D
C81	Myrtenyl acetate	17.06	0.23±0.08	0.02±0.01	0.34±0.16	0.15±0.05	0.03±0.01	0.08±0.01
C46	Nonyl acetate	16.57	N.D	N.D	N.D	N.D	N.D	N.D
C47	*Octyl acetate	14.02	N.D	N.D	0.12±0.01	N.D	N.D	0.07±0
C44	*2-Phenylethyl acetate	15.26	0.24±0.02	N.D	0.17±0.02	N.D	N.D	0.13±0.01
	Total Esters		0.75	0.44	1.02	0.61	0.39	0.7
			Alkenes					
C85	(-)-α-Amorphene	22.4	0.04±0.02	0.01±0	N.D	N.D	N.D	N.D
C7	(E)-β-Famesene	20.22	0.10±0.01	0.02±0	0.09±0.02	0.04±0.01	0.02±0.01	0.05±0.01
C22	*1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	20.54	1.91±0.06	0.90±0.06	1.88±0.25	1.02±0.48	0.71±0.40	1.29±0.25
C41	4-Acetyl-1-methylcyclohexene	12.18	N.D	0.09±0	N.D	0.09±0.01	0.11±0.01	N.D
C83	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	22.3	0.02±0.01	0.01±0	N.D	0.01±0	N.D	0.01±0
C3	*β-Copaene	19.89	2.11±1.76	0.65±0.6	3.07±0.29	0.37±0.56	0.14±0.17	1.35±1.12
C87	*β-Selinene	21.35	0.82±0.02	N.D	0.83±0.1	0.65±0.14	N.D	N.D
C17	γ-Muurolene	20.93	0.17±0.18	0.05±0.04	0.28±0.17	0.13±0.11	0.08±0.04	0.16±0.1
	Total Alkenes		5.17	1.73	6.15	2.31	1.06	2.86
			Alcohols					

C28	1-Hexanol	6.43	N.D	N.D	N.D	N.D	N.D	N.D
C30	1-Tetradecanol	18.72	N.D	N.D	N.D	N.D	0.02±0.02	0.03±0.02
C29	4-Methyl-1-pentanol	5.93	N.D	0.01±0	N.D	0.01±0	0.01±0	0.01±0
C65	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-	18.17	N.D	N.D	N.D	N.D	N.D	N.D
C67	dl-Isopulegol	12.73	N.D	N.D	0.11±0.01	N.D	N.D	N.D
C27	Espatulenol	23.37	0.04±0.01	0.05±0.01	0.03±0.02	0.04±0.03	0.04±0.03	0.04±0
C24	Isoamyl alcohol	4.63	N.D	0.01±0.01	N.D	0.01±0	0.01±0.01	0.01±0
C6	Isoascaridol	16.67	N.D	N.D	N.D	0.08±0.01	N.D	N.D
C92	Phenylethyl Alcohol	11.71	N.D	N.D	N.D	N.D	N.D	N.D
C95	trans-3(10)-Caren-2-ol	16.01	N.D	0.03±0.01	0.04±0	0.09±0.01	0.05±0.02	0.03±0
	Total Alcohols		0.04	0.1	0.18	0.23	0.13	0.12
	Aldehydes							
C72	Heptanal	7.03	N.D	0.03±0.01	0.01±0	0.01±0	0.02±0	0.01±0
C73	*Hexanal	5.43	0.01±0	0.03±0.01	0.01±0	0.02±0	0.02±0	0.01±0
C55	Isovaleraldehyde	3.9	N.D	0.01±0	0.01±0	N.D	0.02±0.01	0.01±0
C88	Octanal	9.03	0.02±0.01	0.01±0	0.01±0	N.D	0.01±0	0.01±0
C26	Perillaldehyde	15.94	N.D	0.04±0.01	0.05±0.01	0.07±0.01	0.08±0.03	0.05±0
	Total Aldehydes		0.03	0.12	0.09	0.1	0.15	0.09
	Ketones							
C48	Acetone	3.02	N.D	0.22±0.03	0.23±0.01	0.31±0.01	0.29±0.05	0.20±0.05

C36	*Piperitone	15.38	0.36±0.06	0.13±0.02	0.41±0.03	0.30±0.02	0.16±0.05	0.35±0.03
C51	Sabina ketone	12.94	N.D	0.22±0.02	N.D	0.20±0.07	0.34±0.05	0.15±0
C93	Salvial-4(14)-en-1-one	23.76	0.01±0	0.01±0	0.01±0	0.02±0	0.01±0	0.01±0
	Total Ketones		0.36	0.57	0.64	0.81	0.79	0.7
	Alkanes							
C5	*(1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo[3.1.0]hexane	11.91	N.D	N.D	N.D	N.D	N.D	N.D
C71	Heneicosane	23.68	0.06±0.01	0.16±0.02	0.05±0.01	0.06±0.03	0.07±0.05	0.06±0.04
C94	Tetradecane	18.92	0.22±0.01	0.48±0.1	0.13±0.03	0.10±0.06	0.26±0.16	0.19±0.03
C96	trans-4-Methoxy thujane	12.67	N.D	N.D	N.D	N.D	N.D	N.D
	Total Alkanes		0.28	0.64	0.18	0.16	0.33	0.25
	Others							
C43	Acetic acid	3.42	N.D	N.D	N.D	N.D	N.D	N.D
C4	Humulene epoxide II	24.16	0.01±0.01	N.D	0.02±0.01	0.06±0.01	0.05±0.01	0.02±0
C91	Oxime-, methoxy-phenyl-_	7.21	0.03±0	0.05±0.01	0.04±0.01	N.D	N.D	0.03±0
C84	Valencen	20.61	N.D	N.D	N.D	N.D	N.D	N.D
C99	Xanthoxylin	25.26	N.D	N.D	N.D	N.D	N.D	N.D
C38	β-Dihydroagarofuran	22.21	0.02±0.02	0.02±0	0.03±0.01	0.02±0	0.02±0.01	0.02±0
	Total Others		2.63	0.56	1.16	0.74	0.41	1.15
Total		53.06	36.4	32.18	30.41		47.18	58.31

Table 3.3 Content (μg/kg) of volatile compounds in the 10 different Red Sichuan Pepper samples, SD: standard deviation. N.D: not detected. RI – Retention Time

Sample			**Red 1**	**Red 2**	**Red 3**	**Red 4**	**Red 5**	**Red 6**	**Red 7**	**Red 8**	**Red 9**	**Red 10**
Code	**Compounds**	**RI**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**	**Avg. ±SD**
						Terpenoids						
C63	β-Elemene	18.81	0.42±0.34	0.19±0.14	0.34±0.46	N.D	N.D	0.13±0.17	0.12±0.08	0.02±0	0.19±0.13	0.29±0.25
C33	*cis-Carveol	14.42	0.05±0.04	N.D	0.13±0.02	0.97±0.23	N.D	0.13±0.02	0.20±0.15	0.11±0.04	N.D	0.19±0.1
C40	*(-)-Terpinen-4-ol	13.53	6.87±0.5	7.92±0.72	11.92±1.28	N.D	9.27±0.33	7.89±0.52	N.D	7.90±1.6	8.25±0.34	N.D
C35	(+)-Isopiperitenone	15.75	N.D	0.02±0.03	N.D	N.D	0.06±0	N.D	0.05±0.04	0.03±0.03	N.D	0.09±0
C52	*(+)-β-Thujone	12	0.14±0	0.13±0.02	0.12±0.1	N.D	0.29±0.04	0.15±0	N.D	0.20±0.01	0.17±0	N.D
C1	*Myrtenal	13.93	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
C2	*Pinene	7.74	N.D	2.36±0.34	3.26±1.12	0.18±0.11	0.39±0.12	2.27±1.05	0.54±0.41	N.D	1.89±1.19	N.D
C20	*(Z)-β-Ocimene	10.02	N.D	2.40±0.82	2.26±1.19	1.36±0.61	1.04±0.14	2.49±0.45	0.87±0.61	3.46±0.9	2.11±0.81	2.81±1.36
C39	2-Acetoxy-1,8-cineole	17.45	0.26±0.04	0.25±0.07	0.31±0.05	N.D	0.26±0.04	0.23±0.04	N.D	0.24±0.02	0.28±0.01	N.D
C32	*2-p-Menthen-1-ol	12.5	0.29±0.34	0.28±0.2	0.29±0.02	0.16±0.03	N.D	N.D	0.36±0.07	0.98±0.05	0.35±0.16	0.75±0.25
C37	*4-Isopropyl-2-cyclohexenone	13.68	0.44±0.01	0.31±0.02	0.52±0.06	0.61±0.1	0.22±0.02	0.34±0.06	0.88±0.23	0.71±0.16	0.28±0.07	0.57±0.1
C42	*4-Terpinenyl acetate	17.3	0.74±0.43	1.80±2.68	0.53±0.08	N.D	N.D	0.69±0.68	0.17±0.02	0.63±0.05	0.32±0.13	0.25±0.06
C54	Bornyl acetate	16.12	N.D	0.23±0.05	N.D	0.09±0.01	0.20±0.02	0.22±0.03	N.D	N.D	0.30±0.04	0.39±0.04
C58	Camphene	8.09	0.08±0.04	0.04±0.01	0.05±0.02	N.D	0.02±0.01	0.03±0.01	N.D	0.09±0.01	0.03±0.02	0.04±0.04
C59	Carveol	14.79	N.D	0.04±0.02	0.06±0.01	0.29±0.06	N.D	0.05±0.02	N.D	0.06±0.02	0.06±0	N.D
C97	Carvyl acetate	17.91	N.D	0.05±0.01	N.D	N.D	0.07±0	N.D	N.D	N.D	N.D	N.D
C60	*Caryophyllene	19.68	1.84±0.29	1.25±0.13	2.15±0.54	0.59±0.29	1.11±0.17	0.99±0.13	0.43±0.17	1.69±0.23	1.31±0.3	1.53±0.5
C61	Caryophyllene oxide	23.54	0.04±0.01	0.02±0.02	0.06±0.01	0.05±0.02	N.D	0.03±0	0.03±0.05	0.05±0.02	0.05±0	0.08±0.01
C62	Citronellal	12.61	0.48±0.1	0.22±0.01	0.37±0.16	N.D	0.11±0	0.16±0.05	N.D	N.D	0.12±0.03	0.14±0.03
C64	δ-Terpineol	13.2	0.17±0.04	0.09±0.01	0.18±0.02	N.D	N.D	0.09±0.02	N.D	0.09±0	0.10±0	0.10±0.02
C16	δ-Terpineol acetate	16.81	0.19±0.03	0.23±0.03	0.32±0.05	0.08±0.01	0.32±0.02	0.21±0.02	0.33±0.05	0.19±0.04	0.25±0.06	0.31±0.06

C66	*δ-Limonene	9.79	N.D	25.56±6.47	19.18±7.61	N.D	18.19±3.49	24.1±3.72	16.02±4.01	N.D	23.69±7.97	N.D
C68	*Eucalyptol	9.94	21.28±1.94	19.28±1.23	22.28±4.61	6.24±1.09	14.7±2.84	17.27±3.5	22.3±2.01	20.53±1.32	19.19±3.28	20.4±2.28
C82	*γ-Cadinene	21.86	0.33±0.05	0.36±0.06	0.71±0.12	0.58±0.13	0.30±0.01	0.24±0.02	0.26±0.13	0.28±0.1	0.33±0.09	0.53±0.18
C69	Geranyl acetate	18.31	0.34±0.04	0.36±0.06	0.39±0.02	0.29±0.02	0.28±0.01	0.29±0.04	0.30±0.02	0.37±0.09	0.46±0.27	0.42±0.14
C23	Germacrene B	23	0.08±0.02	0.25±0.05	0.11±0.03	0.06±0.05	0.34±0.05	0.10±0.02	0.08±0.03	0.04±0.01	0.16±0.07	0.17±0.09
C70	*Germacrene D	21.14	N.D	N.D	1.32±0.27	N.D	0.98±0.15	N.D	0.35±0.26	0.92±0.1	0.87±0.3	0.82±0.31
C77	*Linalool	11.39	13.57±0.17	20.28±1.88	21.02±9.04	27.45±1.95	33.43±1.85	24.08±1.43	23.78±3.24	19.64±0.26	23.29±0.65	18.48±1.79
C78	*Linalyl acetate	15.13	7.72±0.61	N.D	N.D	N.D	0.08±0.01	N.D	N.D	10.26±0.88	N.D	N.D
C79	*Linalyl isobutyrate	15.32	N.D	58.34±4.64	N.D	45.00±2.93	80.99±6.18	42.05±1.93	49.11±4.18	N.D	48.83±5.56	50.07±7.1
C76	*L-α-Terpineol	13.85	3.73±0.46	N.D	4.34±0.37	N.D	N.D	N.D	2.51±0.35	3.03±0.21	N.D	2.95±0.61
C53	*β-Pinene	8.673	1.12±0.66	0.6±0.1	0.51±0.16	0.21±0.12	0.4±0.13	0.46±0.13	0.12±0.03	1.24±0.14	0.41±0.17	0.43±0.38
C31	*Neryl acetate	17.82	0.16±0.02	0.33±0.05	0.22±0.03	0.18±0.01	0.52±0.07	0.27±0.02	0.32±0.04	0.17±0.04	0.33±0.06	0.37±0.06
C90	*o-Cymene	9.64	0.94±0.16	0.76±0.14	1.43±0.18	N.D	0.84±0.19	0.80±0.21	1.27±0.12	3.22±1.95	1.38±0.50	2.61±0.87
C50	*Sabinene hydrate	11.79	N.D	N.D	1.10±1.81	N.D	N.D	N.D	3.64±0.42	1.94±3.27	2.53±2.14	1.79±2.95
C9	α-Cadinol	25.1	N.D	0.04	0.01	N.D	N.D	N.D	N.D	N.D	0.02	N.D
C10	α-Calacorene	22.51	N.D	N.D	0.01±0	0.01	N.D	N.D	N.D	N.D	N.D	0.01
C11	α-Cubebene	17.76	0.09	N.D	N.D	0.17	N.D	N.D	N.D	0.11	N.D	N.D
C12	*α-Phellandrene	9.24	3	1.45	N.D	N.D	N.D	1.48	N.D	2.38	N.D	1.48
C21	*α-Terpinene	9.46	2.67±0.43	2.84±0.22	3.03±1.21	N.D	2.75±0.52	1.87±0.34	1.48±0.42	2.33±0.32	1.69±0.47	2.14±1.09
C13	*α-Terpinyl acetate	17.65	3.89	4.83	5.91	0.61±1	3.99	4.42±0.21	2.05	4.22	4.99	3.99
C49	*α-Thujene	7.561	3.21±1.26	2.03±0.44	1.53±0.41	0.14±0.07	1.32±0.36	1.62±0.37	0.70±0.16	3.32±0.31	1.30±0.57	1.61±1.2
C86	*β-Cadinene	21.94	0.50±0.07	0.58±0.10	1.01±0.18	0.57±0.24	0.45±0.04	0.36±0.02	0.31±0.20	0.41±0.14	0.51±0.16	0.8±0.28
C14	*β-Myrcene	8.759	11.73±2.55	8.52±1.92	8.91±3.44	11.64±4.73	4.76±0.97	8.28±1.29	3.8±1.66	10.23±1.71	7.18±2.34	8.79±4.44
C15	*β-Phellandrene	9.82	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
C18	*γ-Terpinene	10.4	1.99±3.08	1.97±3.13	5.06±4.15	0.10±0.06	4.27±3.72	2.91±2.38	2.44±1.94	5.13±0.68	4.19±0.86	5.17±1.76
C19	τ-Cadinol	24.79	0.01±0.01	N.D	0.02±0	0.01±0	0.01±0	N.D	N.D	N.D	N.D	0.01±0.01
	Total Terpenoids		88.19	163.59	117.51	52.37	181.36	144.23	134.17	106.06	155.22	130.43
							Esters					

C8	(E)-2-Methyl-2-butenyl isobutyrate	5.34	0.01±0	0.02±0.01	0.01±0	N.D	N.D	N.D	N.D	0.01±0	0.02±0.01	0.02±0.01
C34	*6-(Isopropyl)-3-methylcyclohex-2-en-1-yl acetate	16.38	0.54±0.06	0.88±0.14	0.87±0.12	N.D	0.76±0.08	0.69±0.08	0.87±0.09	0.89±0.41	1.27±0.16	1.89±0.18
C25	Isoamyl acetate	6.54	0.07±0.04	0.07±0.01	0.04±0.01	0.04±0.02	0.06±0.02	0.07±0.05	0.04±0	0.06±0.03	0.07±0.04	0.05±0.02
C75	Isobutyl acetate	5.09	0.06±0.03	0.31±0.07	0.04±0.02	0.05±0.01	0.39±0.21	0.12±0.03	0.19±0.02	N.D	0.15±0.1	0.10±0.06
C56	Isobutyl butyrate	8	0.11±0.05	0.04±0.01	0.03±0.01	N.D	N.D	0.02±0.02	0.02±0	0.03±0.01	0.02±0.02	0.05±0.04
C45	*Methyl acetate	3.11	0.37±0.02	0.39±0.08	0.38±0.11	0.68±0.26	0.46±0.03	0.43±0.04	0.34±0.27	0.42±0.11	0.46±0.05	0.72±0.26
C57	Methyl butyrate	4.48	0.01±0	0.01±0.01	0.01±0	0.01±0.01	N.D	0.01±0.01	0.01±0.01	0.01±0	0.01±0.01	N.D
C98	Methyl geranate	16.91	0.03±0.01	0.02±0.01	0.04±0	N.D	N.D	0.02±0.01	N.D	0.04±0.01	0.04±0.01	0.06±0
C74	Methyl hexanoate	7.41	0.02±0	0.04±0.01	0.02±0.01	0.1±0.03	0.03±0	0.06±0.03	0.38±0.1	0.09±0.03	0.09±0.02	0.15±0.05
C89	Methyl octanoate	11.84	N.D	0.03±0	N.D	0.07±0.01	0.03±0	0.04±0.01	0.1±0.02	0.08±0.02	0.06±0.01	0.06±0.04
C80	Methyl valerate	5.77	N.D	N.D	N.D	0.02±0	0.01±0	0.01±0	0.02±0.02	0.01±0	0.01±0	0.02±0
C81	Myrtenyl acetate	17.06	0.10±0.02	0.05±0.03	0.09±0.01	N.D	0.04±0	0.05±0.01	0.03±0.01	0.11±0.04	0.07±0.02	0.10±0.09
C46	Nonyl acetate	16.57	0.07±0.01	N.D	0.07±0	0.07±0	N.D	0.04±0.01	0.03±0.01	0.05±0.02	0.06±0.01	0.08±0.02
C47	*Octyl acetate	14.02	0.81±0.11	0.28±0.04	0.77±0.13	N.D	0.04±0.03	0.31±0.14	N.D	0.48±0.12	0.29±0.09	0.45±0.07
C44	*2-Phenylethyl acetate	15.26	0.20±0.04	N.D	0.29±0.06	0.53±0.17	N.D	N.D	N.D	N.D	N.D	N.D
	Total Esters		2.4	2.14	2.66	1.57	1.82	1.87	2.03	2.28	2.62	3.75
						Alkenes						
C3	*β-Copaene	19.89	0.05±0.02	N.D	0.08±0.01	0.48±0.78	N.D	0.04±0	N.D	0.05±0.01	N.D	N.D
C7	(E)-β-Famesene	20.22	N.D	0.06±0.01	N.D	0.03±0.01	0.07±0.01	0.04±0	0.03±0.01	N.D	0.05±0.02	0.06±0.02
C85	(-)-α-Amorphene	22.4	0.04±0.01	N.D	0.07±0.01	0.05±0.02	0.05±0	0.03±0	N.D	0.03±0.01	0.04±0.01	0.05±0.02
C22	*1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	20.54	0.43±0.09	0.62±0.09	0.70±0.15	N.D	0.91±0.12	0.46±0.01	0.28±0.07	0.39±0.06	0.60±0.15	0.54±0.18
C41	4-Acetyl-1-methylcyclohexene	12.18	N.D	N.D	N.D	0.07±0.06	0.02±0.02	0.01±0.02	0.06±0.01	N.D	0.01±0.02	0.01±0
C83	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	22.3	N.D	0.03±0	0.04±0	N.D	0.03±0	0.02±0	0.01±0.01	0.01±0	N.D	0.03±0.01
C87	*β-Selinene	21.35	0.19±0.16	0.15±0.02	0.44±0.08	N.D	0.15±0.02	0.16±0.01	N.D	0.23±0.05	0.19±0.05	0.16±0.17
C17	γ-Muurolene	20.93	0.23±0.07	0.19±0.03	0.43±0.08	0.18±0.15	0.15±0.01	0.16±0.01	0.06±0.05	0.19±0.05	0.20±0.06	0.28±0.1

	Total Alkenes		0.94	1.05	1.76	0.81	1.38	0.92	0.44	0.9	1.09	1.13
						Alcohols						
C28	1-Hexanol	6.43	N.D	0.02±0	0.03±0.01	N.D	N.D	N.D	0.02±0.01	N.D	N.D	0.03±0.01
C30	1-Tetradecanol	18.72	0.02±0.02	N.D	0.04±0	N.D	0.04±0	0.04±0	N.D	0.02±0.02	N.D	0.04±0.02
C29	4-Methyl-1-pentanol	5.93	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
C65	Cyclohexanol, 2-methylene-3-(1-methylethenyl)-, acetate, cis-	18.17	0.06±0.02	N.D	0.07±0.01	0.05±0.01	N.D	0.04±0	N.D	N.D	0.05±0.01	0.05±0.01
C67	dl-Isopulegol	12.73	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
C27	Espatulenol	23.37	N.D	N.D	N.D	0.06±0.01	N.D	N.D	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
C24	Isoamyl alcohol	4.63	0.01±0.01	0.02±0.02	0.01±0	0.01±0	0.01±0	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.05±0.04
C6	Isoascaridol	16.67	N.D	N.D	0.08±0.01	N.D	N.D	0.05±0.03	0.06±0.04	0.04±0.01	0.07±0.01	0.09±0.02
C92	Phenylethyl Alcohol	11.71	N.D	N.D	0.10±0.01	0.23±0.03	N.D	0.10±0	N.D	0.10±0.02	0.09±0.01	0.11±0.08
C95	trans-3(10)-Caren-2-ol	16.01	N.D	N.D	0.07±0.01	N.D	N.D	0.04±0.02	N.D	0.12±0.05	0.06±0.04	0.13±0
	Total Alcohols		0.09	0.04	0.4	0.35	0.05	0.28	0.12	0.32	0.31	0.52
						Aldehydes						
C72	Heptanal	7.03	0.01±0	0.01±0	0.01±0	0.03±0	0.01±0	N.D	0.03±0	0.01±0	0.01±0	0.01±0
C73	*Hexanal	5.43	0.03±0	0.03±0.01	0.02±0	0.09±0.01	0.02±0.01	0.03±0.01	0.57±0.25	0.02±0	0.02±0	0.04±0.01
C55	Isovaleraldehyde	3.9	N.D	N.D	N.D	N.D	0.03±0.03	N.D	N.D	N.D	N.D	N.D
C88	Octanal	9.03	0.03±0.01	0.02±0.01	0.03±0.02	N.D	0.01±0	0.01±0	0.03±0.01	N.D	N.D	N.D
C26	Perillaldehyde	15.94	0.02±0.01	0.02±0.02	0.05±0.01	0.09±0.03	0.04±0	0.03±0.02	0.04±0.01	N.D	0.02±0.02	0.08±0
	Total Aldehydes		0.09	0.08	0.11	0.21	0.11	0.07	0.67	0.03	0.05	0.13
						Ketones						
C48	Acetone	3.02	0.22±0.02	0.2±0	0.21±0.02	0.37±0.09	0.21±0.03	0.19±0.03	0.27±0.01	N.D	0.18±0.01	0.3±0.07
C36	*Piperitone	15.38	3.01±0.89	0.98±0.55	1.86±0.06	0.21±0.07	0.34±0.01	1.27±0.51	1.13±0.27	4.58±0.42	1.61±0.07	1.82±0.15
C51	Sabina ketone	12.94	N.D	N.D	N.D	N.D	N.D	N.D	0.18±0.07	0.08±0	N.D	0.09±0.03
C93	Salvial-4(14)-en-1-one	23.76	N.D	N.D	0.01±0	0.01±0	N.D	N.D	0.01±0	0.01±0	0.01±0	0.01±0
	Total Ketones		3.23	1.18	2.08	0.59	0.55	1.46	1.59	4.67	1.8	2.22
						Alkanes						

C5	*(1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo[3.1.0]hexane	11.91	0.33±0.03	0.34±0.05	0.67±0.1	N.D	0.51±0.08	0.28±0.02	0.33±0.06	0.29±0.01	0.27±0.03	0.28±0.09
C71	Heneicosane	23.68	N.D	0.01±0	0.02±0.01	N.D	0.01±0	N.D	0.01±0	N.D	N.D	0.02±0.01
C94	Tetradecane	18.92	0.09±0.02	0.07±0.01	0.11±0.02	0.08±0.03	0.08±0.03	0.07±0.01	N.D	0.09±0.01	0.08±0.01	0.08±0.03
C96	trans-4-Methoxy thujane	12.67	N.D	0.10±0.01	0.21±0.01	N.D	0.09±0.01	0.09±0.03	N.D	0.18±0.04	0.15±0.01	0.27±0.06
	Total Alkanes		0.42	0.42	1.01	0.08	0.69	0.44	0.34	0.56	0.5	0.65
							Other					
C43	Acetic acid	3.42	0.34±0.1	0.22±0.19	N.D	0.25±0.12	0.24±0.08	0.17±0.05	0.37±0.1	N.D	0.28±0.14	0.26±0.22
C4	Humulene epoxide II	24.16	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.01±0	0.02±0	0.01±0	0.01±0	0.01±0
C91	Oxime-, methoxy-phenyl-_	7.21	0.02±0	N.D	0.02±0.01	N.D	N.D	0.02±0.01	N.D	0.02±0	0.02±0	N.D
C84	Valencene	20.61	0.40±0.23	0.05±0.05	0.10±0.1	N.D	N.D	N.D	N.D	N.D	0.10±0.07	0.07±0.09
C99	Xanthoxylin	25.26	0.02±0.01	N.D	N.D	N.D	N.D	N.D	N.D	0.08±0.03	0.01±0	N.D
C38	β -Dihydroagarofuran	22.21	N.D	N.D	N.D	0.02±0	N.D	N.D	N.D	N.D	N.D	N.D
	Total Others		0.79	2.64	3.39	0.46	0.64	2.47	0.93	0.11	2.31	0.34
Total		96.15	171.14	151.74	114.93	140.29	128.92	186.6		163.9	139.17	56.44

All compounds names are referred to PubChem. (https://pubchem.ncbi.nlm.nih.gov/)

* means the compound was applied to PLSR analysis.

Clustering of Sichuan Peppers

The Green Sichuan Pepper samples clustered different to Red Sichuan Pepper samples in general (figure 3.1). There were 24 volatile compounds only found in Red Sichuan Pepper. These included 11 terpenoids which were α-Cadinol, α-Calacorene, α-Phellandrene, 2-Acetoxy-1,8-cineole, Linalool, Germacrene D, Geranyl acetate, Eucalyptol, δ-Terpineol, Carvyl acetate, Linalyl isobutyrate; 3 alcohols, which were 1-hexanol, phenylethyl alcohol, and cis-2-methylene-3-(1-methylethenyl)-Cyclohexanol acetate; 5 esters, which were 6-(isopropyl)-3-methylcyclohex-2-en-1-yl acetate, isobutyl acetate, isobutyl butyrate, methyl valerate, and nonyl acetate; 2 alkanes, which were (1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo[3.1.0]hexane and trans-4-methoxy thujane; 3 other chemical families, which were acetic acid, xanthoxylin and valencen. There were 3 volatile compounds only found in Green Sichuan Pepper samples, which were Myrtenal, β-Phellandrene, and 4-Methyl-1-pentanol. To compare with AHC clustering of Sichuan Peppers by sensory attributes (figure 2.2), the Red 1 sample kept away from the Green Sichuan Peppers by clustering of volatile compounds, which was different from clustering of sensory attributes (figure 2.2). It was because of the total volatile compounds contents of Red 1 are greater than each of Green Sichuan Pepper samples, in which we didn’t find Eucalyptol and Linalool in Green Sichuan Pepper samples. These two volatiles compounds take a big ratio in total contents of Red Sichuan Pepper samples, which lead the clusters to be different.

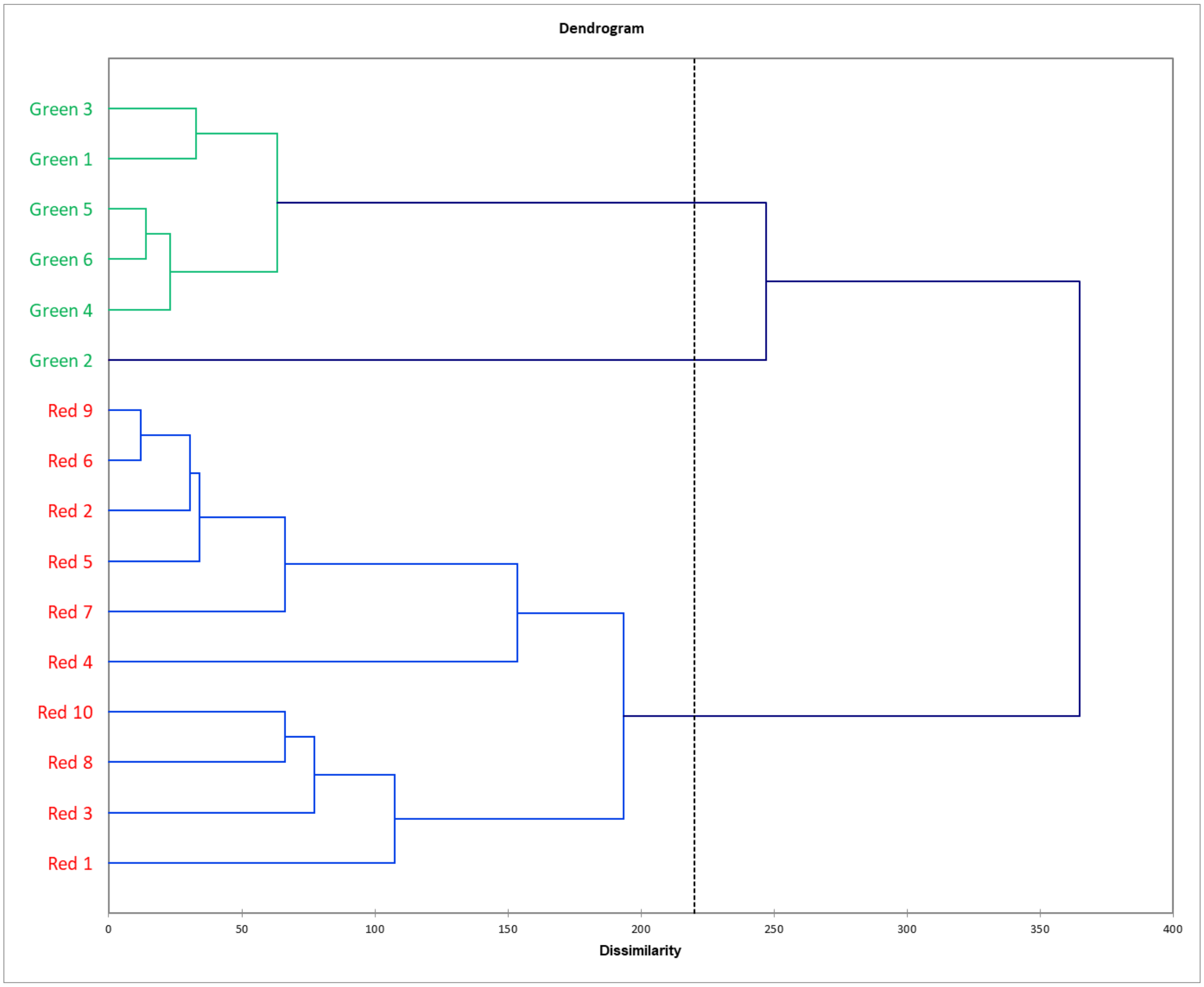

Figure 3.1 Agglomerative hierarchical clustering (AHC) of chemical compounds for Sichuan Pepper samples

Sensory Attributes of Samples

The aroma and flavor characteristics of the Sichuan pepper samples have been described in detail in Chapter 2 Results section.

Green Sichuan Pepper samples had slightly higher average intensity scores for all significantly different ($P \leqslant 0.05$) aroma and flavor attributes than Red Sichuan Pepper samples.

When testing products in similar type or category, it would be expected that the products would have similar characteristics. Within the Green Sichuan Pepper samples, all sensory attributes didn't show significant difference ($P \leqslant 0.05$) for aroma profile, and only attribute of tingle showed a significant difference in flavor profile. Also, we would expect Red Sichuan Pepper samples to have similar characteristics, but 16 out of 32 attributes were detected significantly different ($P \leqslant 0.05$). It could be explained by different growth conditions, genetic characteristics, processed methods and ages caused the major chemical different (Wang et al., 2010; Li et al., 2014). Total of 18 sensory attributes were detected significant differences between Red and Green Sichuan Pepper samples: a) aroma: Peppery, Green, Pungent, Anise Fennel, Piney, Woody, Fruity and Chive, and b) flavor: Green, Floral, Pepper, Green Viney, Pungent, Piney, Bitter, Tingle Tongue, and Numbness. Those results showed that the reported sensory attributes would be useful to identify Sichuan Pepper samples.

PLSR was adopted to show the association between sensory attributes and volatile compounds, which is shown as below.

Association of Sensory Attributes and Volatile compounds:

The first two partial least squares factors explained 77 % of the X-matrix (instrumental data) variability and 68% of the Y-matrix (descriptive data) variability (Figure 3.3).

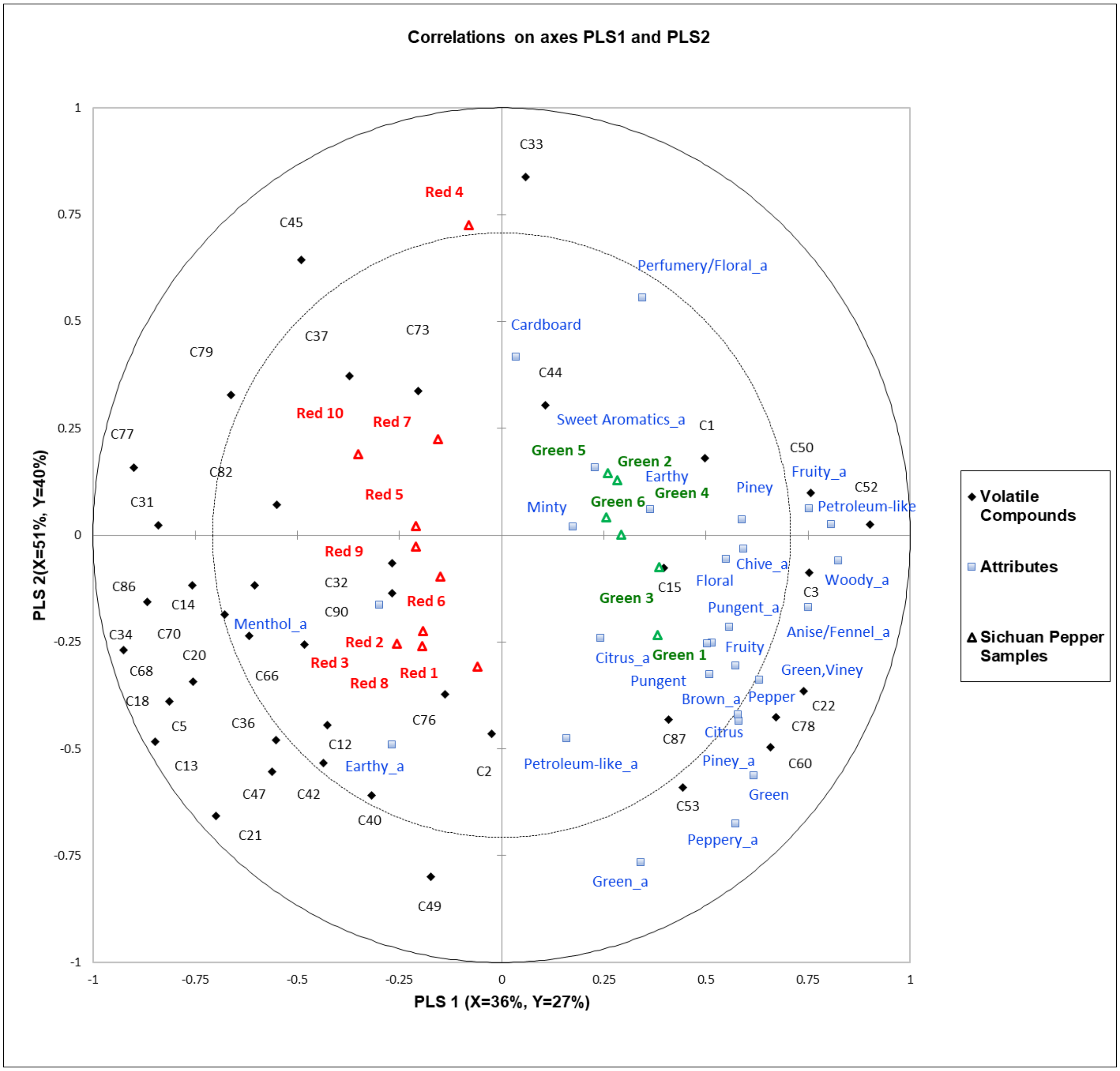

Figure 3.2 Partial least squares regression factors 1 (x = 36%, y = 27%) and 2 (x = 51%, y = 40%). X-matrix = chromatographic analysis data and Y-matrix = descriptive sensory data. Black dots (C): chemicals from the chromatographic analysis; Blue squares: sensory attributes from the descriptive sensory data (no suffix: flavor; a suffix: aroma). Red triangles: Red Sichuan Pepper; Green triangles: Green Sichuan Pepper.

β-Copaene (C3) was correlated to fruity aroma, anise fennel aroma, and petroleum-like flavor ($r \geq 0.6$). Oyedeji & Afolayan (2005) reported it as one of important compounds in the essential oil of pennywort. FooDB (2019) reported it as a potential biomarker in several food items, such as sage, peppermint, and corn. The correlation ($r \geq 0.6$) between petroleum-like flavor and anise fennel aroma in the PLS model provided pieces of support about the relationship between C3 and petroleum-like flavor, and anise fennel aroma. Caryophyllene (C60) was correlated to both anise fennel aroma ($r \geq 0.6$) and green flavor ($r. \geq 0.6$). Its profile was spice, clove, woody, herbal, sweet, nutmeg, and peppery. It was common detected in spices such as cloves, ginger, rosemary, and thyme (Perfumer & Flavorists, 2019).

α-Phellandrene (C12) was related to earthy aroma ($r \geq 0.6$). It is an important volatile compound found in white and black pepper (Jagella et al., 1999), which flavor described as peppery-minty, slightly citrusy, herbal, terpenic, green and woody. Mixture of α-Phellandrene with α-Thujene (C49) and 2-octanone in some ratio would present earthy flavor (The Good Scent Company, 2019).

cis-Carveol (C33) and 2-Phenylethyl acetate (C44) were both related to perfumery floral aroma ($r \geq 0.7$) in this model. The odor and flavor of cis-Carveol was identified as spearmint and caraway (The Metabolomics Innovation Centre, 2019). The odor of 2-Phenylethyl acetate was described as sweet rose and honey aroma (Surburg et al., 2016). The definition for perfumery floral aroma described in Chapter 2, Table 2.3, as 'Floral aromatics with somewhat sweet, non-natural notes not generally associated with fresh fruit.' The sensory attributes would good to include characteristic of this volatile compound.

Sabinene hydrate (C50) was correlated to woody aroma also, and petroleum-like flavor ($r \geq 0.6$). From the literature report (Perfumer & Flavorist, 2019), Sabinene hydrate had aroma of mandarin, peppermint and rosemary etc. in natural occurrence; also had woody, citrus, orange and grapefruit aroma in 5 ppm. In the PLS model, woody aroma correlated to petroleum-like flavor ($r \geq 0.8$), which would be a piece of support the associations between Sabinene hydrate and woody aroma and petroleum-like flavor.

(+)-β-Thujone (C52) was correlated to anise fennel aroma, fruity aroma, woody aroma, green flavor, petroleum-like flavor, and green viney flavor. This volatile compound varied flavor and aroma in different concentrations from 1% to 10%. The flavor of it reported as herbal, cedar, thujonic, spicy, woody and warm. The mixture of (+)-β-Thujone and α-Cadinol (C9) would provide strong herbal flavor (The Good Scent Company, 2019). In the correlation matrix, these sensory attributes also showed correlation to each other ($r \geq 0.65$).

β-Pinene (C53) was related to peppery aroma ($r \geq 0.6$) and citrus flavor ($r \geq 0.6$) in this model. Its aroma profile was reported as woody, turpentine, terpy, green, citrus, pine, and cooling (Perfumer & Flavorists, 2019). It existed in many natural spices such as anise seed, basil, caraway, cardamom, dill, and nutmeg.

δ-Limonene (C66) was related to menthol aroma in the model (($r \geq 0.7$). The aroma and flavor characteristics of this volatile compound usually described as citrus, orange, terpy, and spice herbal (Perfumer & Flavorists, 2019). It is a very common material, especially in orange oil.

Linalyl acetate (C78) was correlated to peppery flavor attribute and anise fennel aroma attribute ($r \geq 0.6$). It was reported as citrus fruits, thyme, wine, lavender etc. in natural occurrence. In 1% dilution range, it still had citrus floral, lavender and green aroma. It maintained these characteristics to 15 ppm dilution (Perfumer & Flavorists, 2019).

According to Figure 3.2, the Green Sichuan Pepper samples were more correlated to these sensory characteristics than Red Sichuan Pepper samples. But it did not imply that Red Sichuan Pepper was not appropriated by these sensory characteristics. It just inferred the result of comparison between Green and Red Sichuan Pepper samples. For example, linalool (C77) was also reported as an important aroma component for Sichuan Pepper and present strong floral flavor (Yang., 2008). But it situated away from floral aroma and flavor in Figure 3. Furthermore, there were still several attributes and volatiles lack of direct relationship. It may be that in flavor matrices, the combined chemicals were more related to sensory characteristics than single chemical. Also, in the GC-MS results, the total quantity of volatile compounds detected in Green Sichuan Pepper samples were less than Red Sichuan Pepper samples. It might be the samples were ground powder but not the essential oil. The chemicals of Green Sichuan Pepper might be difficult to extract out.

Limitation

There are a few limitations in this study should be addressed for future studies. Firstly, the 16 Sichuan Pepper samples applied int his study were carefully selected, but they can't cover the full ranges and subspecies in all Sichuan Peppers, such as species of *Zanthoxylum americanum* Mill., a specialty variety in the US. We knew the Red Sichuan Pepper samples had longer ages than Green Sichuan Pepper samples, but we didn't know exactly ages of those samples. The future studies might include more species of *Zanthoxylum* genus and more details for these species, such as ages, processing methods, etc. Also, to reach more association between sensory characteristics and chemical compounds, applying more instrumental analysis would be helpful. For example, High-performance liquid chromatography, would be helpful to detect Alkylamides for numb and tingle principles; Gas Chromatography Analysis with Olfactometric Detection, would be helpful to identify more details of relationship between single chemical and descriptors

Conclusions

In this study, total of 99 different volatile compounds were detected and semi-quantified in 6 samples of Green Sichuan Pepper and 10 samples of Red Sichuan Pepper. The 46 terpenoid compounds detected were the most abundant volatile compounds. Partial least squares regression was used to associate descriptive sensory characteristics to volatile compounds. Possible relationships between volatile compounds and sensory attributes were detected, in which the most prominent associations included β -Copaene related to Anise/Fennel aroma and Fruity aroma, and Petroleum-Like flavor, α-Phellandrene related to Earthy aroma, cis-Carveol and 2-Phenylethyl acetate related to Perfumery/Floral aroma, Sabinene hydrate related to Woody aroma and Petroleum-Like flavor, (+)-β-Thujone related to Anise/Fennel aroma, Fruity aroma, Woody aroma, Green flavor, Petroleum-Like flavor, and Green Viney flavor, β-Pinene related to Peppery aroma and Citrus flavor, δ-Limonene related to Menthol aroma, Linalyl acetate related to Pepper flavor, and Anise Fennel aroma. Results suggested age would be an important factor to differentiate Sichuan pepper. However, this study tested many market samples differed in their ages with but limited in number of categories. It didn't include some specialty varieties, such as species of *Zanthoxylum americanum* Mill. in the US. The future studies might include more species of *Zanthoxylum* genus to generate more detailed sensory characteristics descriptors. Also, it would be worthy to apply other kinds of instrumental analysis as well, such as High-performance liquid chromatography, to detect Alkylamides for numb and tingle principles, Gas Chromatography Analysis with Olfactometric Detection, to identify more details of relationship between single chemical and descriptors. It would be helpful to uncover the deep relations between volatile compounds and descriptors of Sichuan Pepper samples.

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Chapter 4 - Conclusion and Future Research

In this research, descriptive analysis of sensory profiles and instrumental analysis for different subspecies of Sichuan pepper has been conducted in order to investigate the Lexicon development of *Zanthoxylum* genus, also known as Sichuan pepper, associated with volatiles.

Total of 32 sensory attributes were detected by panelists in sensory analysis part of this study as shown in Table 2.2. The main characteristics of Sichuan Pepper samples are Peppery attribute for both aroma and flavor profile, Green attribute for both aroma and flavor profile, Tingle Tongue attribute for mouthfeel and Numbness attribute for mouthfeel. Among which, 18 attributes were detected significantly different for the Green Sichuan Pepper samples and the Red Sichuan Pepper samples). For aroma profile, attributes were Peppery, Green, Pungent, Fennel Anise, Piney, Woody, Fruity, and Chive. For flavor profile, attributes were Green, Peppery, Petroleum-like, Floral, Green Viney, Pungent, Piney, Bitter, Tingle Tongue, and Numbness.

Furthermore, total of 99 different volatile compounds were detected and semi-quantified in 6 samples of Green Sichuan Pepper and 10 samples of Red Sichuan Pepper. The 46 terpenoid compounds detected were the most abundant volatile compounds. Possible relationships between volatile compounds and sensory attributes were detected through partial least squares regression, in which the most prominent associations included β-Copaene related to Anise/Fennel aroma and Fruity flavor,α-Phellandrene related to Earthy aroma, cis-Carveol and 2-Phenylethyl acetate related to Perfumery/Floral aroma, Sabinene hydrate related to Woody aroma and Petroleum-Like flavor, (+)-β-Thujone related to Anise/Fennel aroma, Fruity aroma, Woody aroma, Green flavor, Petroleum-Like flavor, Green Viney flavor, Pungent flavor, and Bitter flavor, β-Pinene related to Citrus flavor, Caryophyllene related to Anise/Fennel aroma, δ-Limonene related to Menthol aroma, Linalyl acetate related to Pepper aroma, Fruity aroma and Citrus flavor, β-Selinene related to Pepper flavor and Petroleum-Like flavor.

There are a few limitations in this study should be addressed for future studies. Firstly, Sichuan Pepper samples were evaluated in boiled water, in which situation, some chemicals might be destructed before sensory evaluation. The characteristic chemical components of Sichuan Pepper are alkaloids and terpenoids, which provided the tingle, numb and floral sensation. In distilled water, the high temperature might evaporate the chemical from water in reducing the intensity of perception. Immersing the Sichuan Pepper samples in sunflower oil and evaluating the extraction oil could be a solution, however, the limitation is that the extraction oil would be easily oxidized, and the sensory profile could be changed. Furthermore, the 16 Sichuan Pepper samples used in this study were carefully and widely chosen, while they cannot cover the full ranges and subspecies in all Sichuan Peppers. This result of instrumental analysis suggested that age would be an important factor to differentiate Sichuan pepper. However, this study tested many market samples differed in their ages with but limited in number of categories. It didn't include some specialty varieties, such as species of *Zanthoxylum americanum* Mill. in the US. The future studies might include more species of *Zanthoxylum* genus to generate more detailed sensory characteristics descriptors. Also, applied more instrumental analysis, such as High-performance liquid chromatography, to detect Alkylamides for numb and tingle principles, Gas Chromatography Analysis with Olfactometric Detection, to identify more details of relationship between single chemical and descriptors. It would be helpful to uncover the deep relations between chemicals and descriptors of Sichuan Pepper samples.