IDENTIFICATION AND CONCENTRATION OF PHENOLIC AND CARBONYL COMPOUNDS IN FLORIDA HONEYS

By

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To my family, Marty, Julie, Melissa, Kelly, Kristin, and Ruby, you have all been there to support, love and encourage me in all my wild endeavors.

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LIST OF ABBREVIATIONS

AAPH	2,2’-azotis(2-amidinopropane)
DAD	Diode array detector
DPPH	2,2-diphenyl-1-picrylhydrazyl
g	Gram
GAE	Gallic acid equivalents
h	Hour (s)
HPLC	High performance liquid chromatography
L	Liter
µg	Microgram
µl	Microliter
µmol	Micromole
min	Minute (s)
ml	Milliliter
mm	Millimeter
MS	Mass spectrometer
m/z	Mass to charge ratio
nm	Nanometer
OPD	Ortho-phenelyenediamine
ORAC	Oxygen radical absorbance capacity
psi	Pounds per square inch
rpm	Revolutions per minute
TE	Trolox equivalents
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
UV	Ultraviolet

v	Volume
Vis	Visible
w	Weight

Abstract of Thesis Presented to the Graduate School
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Honeys contain phenolic compounds and α-dicarbonyls with antioxidant and anti-microbial capacities, respectively. The type and concentration of these compounds vary depending on the floral source and geographical location where the honey is produced. Forty-three varietal honeys, including 26 monofloral and 17 multi-floral honeys were sampled from different regions of Florida. The monofloral honeys included those from orange blossom, mangrove, tupelo, palmetto, Brazilian pepper, blueberry, blackberry, gallberry, avocado, and white clover. These honeys were evaluated for their antioxidant capacity, total phenolic content, and free radical scavenging capacity. Phenolic phytochemicals and α-dicarbonyls were identified and quantified using HPLC-DAD-MS^n. Avocado honeys had a total phenolic content of 1,570 μg GAE/ml, which was higher than all other Florida varieties and certified Manuka honeys. White clover honey showed the lowest total phenolic content of 250 μg GAE/ml. The free radical scavenging capacities were tested using the Oxygen Radical Absorbance Capacity assay (ORAC). The ORAC values of the honeys ranged from 1.50-28.0 μmol TE/g. Tupelo, avocado and a multi-floral honey showed the highest ORAC values. All honeys contained 3-deoxyglucosone at a higher concentration than methylglyoxal or glyoxal.

Manuka honeys had higher concentrations of methylglyoxal than other varieties. Chrysin, 2-*cis*, 4-*trans* and 2-*trans*, 4-*trans*-abscisic acid, pinocembrin, and pinobanksin were found in nearly all honeys examined. The plant hormone 2-*cis*, 4-*trans*-abscisic acid was found at the highest concentration in many of the honeys.

CHAPTER 1
INTRODUCTION

Honey

There has been a heavy push for healthy eating habits and a demand for an increase in consumption of fruits and vegetables due to their potential health benefits. Honey is not considered to be a fruit or vegetable; it is almost completely composed of carbohydrates. What people often overlook is how honey is made. Honey is produced from honey bees using the nectar from plants.[1] Honey can generally be classified by season, geographical location and botanical source.[2] Honey adulteration has been a major problem in the honey industry. To prevent adulteration from happening, a simple method of determining the pollen type and concentration has been utilized to confirm botanical source.[3] This is important because research suggests that the main floral source of the honey may have the most significance on the medicinal properties of the honey.

Research on honey spiked with the discovery of New Zealand Manuka honey. This honey claims to have higher antimicrobial capacity unique to the variety that is used in the treatment of infections and other types of wounds. Now, research in the field has begun to focus on what the component(s) are in this Manuka honey that give it these antimicrobial properties, and if honey from other floral and regional sources will have similar properties. It is known that certain phytochemicals in honey have health-promoting effects by acting as natural antioxidants, anti-tumorigenic, and anti-carcinogenic agents, which, in turn, reduces the risk of cardiovascular disease, cancer, immune-system decline, cataracts, and different inflammatory processes.[4] The goal of current research is to quantify and identify the components in various honey samples to

show which have these health-promoting benefits and what the bioactive components are.

Phenolic Phytochemicals

Phenolic phytochemicals are the secondary metabolites of plants. The identification and quantification of phytochemicals is necessary in determining the potential health benefits of honeys. It has been previously determined that the bioactive phytochemicals in honey that account for the honey's antioxidant capacity are from the flavonoid and phenolic acid groups. In order to retrieve these compounds, an extraction process is necessary. Most research has used Solid Phase Extraction (SPE) to eliminate all other components except the bioactive compounds of interest. In an experiment looking at the isolation and identification of phenolic acids in Malaysian honey, the honey samples were absorbed onto preconditioned C18 column cartridges.[5] Before this technique was developed, the main way to extract the phenolic compounds was by passing the samples through a column of Amberlite XAD-2 resin.[6] The ultimate goal of both the above methods is to keep the phenolic and flavonoid compounds on the column while eluting any polar compounds present, like sugar. The potential problem with using these methods without any modification is that not all the bioactive compounds are retained, and it is near impossible to remove all of the other material from the sample, which may interfere with later results.[7] The benefit of using the C18 column is they are easily available in small disposable cartridges. However, an experiment testing both techniques showed that though the C18 cartridge gave a higher rate of flavonoid recovery, it was less appropriate for the isolation and extraction of phenolic acids.[3] Though all of these methods are proven to isolate phenolic acids and flavonoids, there is evidence that for specific compounds, such as glycosides, the C18

cartridges are more effective in compound retention than the nonpolar XAD-2 resin.[8] Therefore, it may be useful to carry out different extraction techniques depending on the compound of interest.

Once the isolation of the bioactive materials has been completed, a series of tests can be performed to get a better understanding of the possible antimicrobial and health benefits honeys may possess. Since honey is being studied as a possible natural antioxidant researchers can perform a few tests to determine antioxidant capacity and radical scavenging capabilities. For an estimation of total phenolic content, which has proven to have a strong correlation with overall antioxidant activity, the Folin-Ciocalteu method is the standard method in use.[9] This method does not differentiate between phenolic compounds and further analysis is needed in order to identify the different phenolic phytochemicals. In a study that analyzed total phenolics in Burkina honey, the total phenolics observed ranged from 32.59 to 114.75 mg/100g of honey.[10] These values are different than the values obtained from another study investigating Slovenian honeys, which obtained total phenolic values ranging from 44.8mg to 241.4mg/100g of honey.[11] These differences can be explained by the different geographical locations and floral source of the honey samples. Values from other studies show similar variance between total phenolics.

There are two primary tests to determine the antioxidant properties of honey, the DPPH assay and the ORAC assay. The DPPH radical scavenging assay is useful in investigating the overall hydrogen/electron donating activity of single antioxidants.[12] The ORAC assay measures the ability of honey to scavenge peroxyl radicals.[13] Again, these values vary between studies due to the floral source, geographical location and

storage conditions the honey is produced and maintained. One test used *in vitro* conditions to test antioxidant activity of honey samples, using guinea pig liver homogenate.[14] This technique had never been used before for honey analysis and has not been repeated since, even though it showed a direct, positive correlation between the total phenolic content and the antioxidant capacity. There has also been an association with honey color and antioxidant activity. It is suggested that the darker the color of the honey, measured by spectrophotometry methods, the higher the antioxidant activity.[15] Though it is suspected that the color is influenced by the type and concentration of the phenolic compounds present in the sample, there are other factors in honey that may affect the color, such as storing conditions and concentration of other components in the honey.

High performance liquid chromatography (HPLC) has been commonly used as a way to quantify compounds. In honey these are most commonly the flavonoid and phenolic compounds, but some research analyzes the carbohydrate components as well. HPLC performed with mass spectrometry (MS) is used to be able to quantify and tentatively identify the compounds in a sample of honey. HPLC/MS allows for specific, single compounds to be quantified and identified whereas the previous tests do not. This test is crucial for an experiment to have conclusive data to create an association with the components of honey and their potential health benefits and antimicrobial properties. Table1-1 summarizes a majority of the phenolic phytochemicals previously identified various honey sources. The table shows that floral source can greatly influence the phytochemical profile of honeys.

There is an abundance of research proving that phenolic acids and flavonoids have antioxidant capabilities. However, different compounds have proven to show more capacity and therefore it is necessary to be able to quantify and identify the compounds in order to be able to properly attribute the behavior. The quantification is also important because there has been research to prove that the higher the concentration of these compounds the more antioxidant capacity they usually possess. All of these tests are important in determining the potential beneficial uses of honey as more than just a sweetener.

Phenolic acids and flavonoids are known to be high in antioxidants. The higher the concentration of these compounds in honey, the higher the sample's antioxidant capacity, and the higher the added health benefits, such as prevention of oxidative stress and scavenging of free radicals.

Antimicrobial Activity

There has been considerable debate on what the source of the antimicrobial property is associated with some honey varieties. The most established research focuses on looking at the components of Manuka honey, because it has been shown to inhibit the growth of certain bacterial strands.[16] Manuka honey is often used as a comparison to match components from its own composition with honey from other floral and geographical sources. First, however, the antimicrobial component in Manuka needed to be isolated and determined. All honeys are known to contain peroxides (from natural reactions within the sample), which are associated with some antibacterial properties, though not at the magnitude that Manuka honey exhibits.[17] There is evidence that Manuka has antibacterial properties not associated with the peroxides in the honey, which has been determined after the peroxides have been removed by

catalase.[18] Some research attributes the added antibacterial property of the Manuka and other honeys to the phenolic acids present, while others suggest the primary antibacterial component is a dicarbonyl compound called methylglyoxal.[19-20]

The idea was tested by measuring the potency of the antibacterial activity in a variety of samples.[21] Generally if inhibition is shown further analysis is done to determine the composition of the honey that can be attributed to the antibacterial property, usually through HPLC/MS as described previously. Once the compounds are identified, samples of that compound could then be used in similar methods to positively identify that the compound indeed exhibits antibacterial capabilities. The majority of research has concluded that the main source of non-peroxide antibacterial properties come from the α-dicarbonyls, glyoxal and methylgloxal, in certain honey.[22] The reactive carbonyl compounds in honey are formed by carbohydrate degradation, similar to the Maillard Browning reactions.[17, 22] The higher the concentration of methylglyoxal the more microbial inhibition has been seen.[16, 18, 23] The concentration of these α-dicarbonyls cited from previous research can be seen in Table 1-2. As stated Manuka honeys, thus far, have been shown to contain significantly higher levels of methylglyoxal than other varieties. The problem with this compound is that it is reactive and may cause further oxidation in the body.

The use of honey for topical use has expanded from the discovery of its antibacterial properties. Honey has shown to inhibit to some extent *Staphylococcus spp.*, *E. Coli*, *Salmonella spp.*, and *streptococcus spp.* among others proving it can be used as a viable medical treatment.[24] These antibacterial properties have been extensively studied in Manuka honey, but need to be further investigated in other types

of honey to definitively determine the compound responsible for antimicrobial resistantce. Table 1-3 gives a brief summary of some of the microbes honeys have shown to have some activity against. The most common tested microorganism was *Staphylococcus aureus* which honey showed to have moderate inhibition against.[25]

Summary

The research on honey focuses on the food's chemical composition and its potential use in the medical industry. Researchers constantly try to invent better methods of analyzing the phytochemicals that are responsible for the antioxidant properties seen in a wide variety of honeys from tests like ORAC and DPPH. Further studies need to be investigated to see the extent of honey's antioxidant capacity in order to assess the benefits in living cells and organisms.

The antimicrobial properties in honey are an even bigger research area, because it may prove to be an inexpensive, effective medical treatment for wound victims. It has been determined that there are multiple components of honey that contribute to this antibacterial phenomenon. A larger sampling from different areas and a variety of floral source should be assessed to determine if any other types of honey exhibit the properties of the well-researched Manuka honey.

Research Objectives

This research was to compile comprehensive knowledge of phenolic and α-dicarbonyls compounds in Florida honeys and to investigate the antioxidant capacity of different varieties. The specific objectives were:

1. To evaluate antioxidant capacity, phenolic content, and color in Florida and Manuka honeys.
2. To investigate the α-dicarbonyl and phenolic phytochemicals present in Florida and Manuka honeys using HPLC-DAD-ESI-MS^n.

Table1-1. Commonly reported phytochemicals in multi- and monofloral honeys.

Phytochemicals	Total Concentration range (µg/g)	Type of honeys	Reference
Gallic acid	0.8-2372	Coconut, Gelam, Australian Jelly Bush, Manuka, heather, linden, lavender, Kanuka,	1, 5-6, 26-27
Caffeic Acid	0.22-18.4	Coconut, Gelam, Australian Jelly Bush, Manuka, Linen vine, morning, glory, black mangrove, singing bean, Christmas vine, lavender, heather	1, 5-6, 26, 28-29
Benzoic Acid	0.797-1.84	Coconut, Gelam	5
Hydroxybenzoic acid	0.24-62.1	Heather, linden, buckwheat, soy, clover, fireweed, acacia	1, 6, 9, 29
Ferulic acid	0.23-58.6	Gelam, Australian Jelly Bush, Manuka, Linen vine, morning, glory, Christmas vine, heather, buckwheat	5-6, 26, 28-29
Cinnamic acid	0.19-5.4	Gelam, lavender, heather, buckwheat, Hawaiian Christmas berry, soy, tupelo, clove, fireweed, acacia	5-6, 9
Chlorogenic acid	0.7-33.4	Australian Jelly Bush, Manuka, lavender, heather, buckwheat	6, 26, 29
Coumaric acid	0.1-47.4	Australian Jelly Bush, Manuka, Linen vine, morning, glory, black mangrove, singing bean, Christmas vine, lavender, heather, buckwheat, soy, clover, fireweed	6, 9, 26, 28-29
Ellagic acid	0.4-27.0	Australian Jelly Bush, Manuka, heather, buckwheat	6, 26, 29
Syringic acid	0.1-4.6	Australian Jelly Bush, Manuka, Linen vine, morning, glory, black mangrove, heather, linden, clover, Kanuka	6, 9, 26-29
Vanillic acid	0.1-30.3	Linen vine, morning, glory, black mangrove, singing bean, Christmas vine, lavender, heather, buckwheat, tupelo, clover	1, 6, 9, 28-29
Rosmarinic acid	0.19-15.1	Buckwheat, heather	29
Phenyllactic acid	20-1900	Manuka, kanuka	27
Methyl syringate	1.7-207	Manuka, kanuka	27
Pinobanksin	0.1-15.6	Australian Jelly Bush, Canola, cherry, eucalyptus, lavender, linden, lucerne, orange, rapeseed, rhododendron, rosemary, taraxacum, tilia, sunflower, Spanish multi-floral, buckwheat, soy, tupelo, clove, fireweed, acacia	7, 9, 26, 30-31

Table1-1. contd

Phytochemicals	Total Concentration range (µg/g)	Type of honeys	Reference
Myricetin	0.3-19.1	Australian Jelly Bush, Manuka, singing bean, eucalyptus, buckwheat, heather	26, 28, 30
Tricetin	0.7-6.8	Australian Jelly Bush, eucalyptus	26, 30
Quercetin	0.05-11.9	Australian Jelly Bush, Manuka Canola, eucalyptus, lucerne, orange, rapeseed, rosemary, taraxacum, tilia, sunflower, linden, heather, Spanish multi-floral, Melon, pumpkin, cherry, dandelion, maple, pinetree, buckwheat, soy, clover, acacia	1, 7-9, 26, 29-31
Luteolin	0.03-5.7	Australian Jelly Bush, Manuka, canola, eucalyptus, lavender, linden, orange, rhododendron, taraxacum, Spanish multi-floral, rosemary, Melon, pumpkin, cherry, dandelion, maple, pinetree	7-8, 26, 30-31
Kaempferol	0.1-3.9	Australian Jelly Bush, Manuka, Linen vine, morning, glory, black mangrove, singing bean, Christmas vine, Canola, cherry, eucalyptus, lavender, linden, lucerne, orange, rapeseed, rhododendron, rosemary, taraxacum, tilia, sunflower, linden, heather, Spanish multi-floral, Melon, pumpkin, cherry, dandelion, maple, pinetree, buckwheat, soy, tupelo, clove, acacia	1, 7-9, 26, 28-31
Apigenin	0.03-2.1	Spanish multi-floral, rosemary	7, 31
Pinocembrin	0.12-15.6	Australian Jelly Bush, Manuka, Canola, cherry, eucalyptus, lavender, linden, lucerne, orange, rapeseed, rhododendron, rosemary, taraxacum, tilia, sunflower, Spanish multi-floral buckwheat, soy, tupelo, clove, fireweed, acacia	7, 9, 26, 30-31
Chrysin	0.06-4.0	Australian Jelly Bush, Manuka, Canola, cherry, eucalyptus, lavender, linden, lucerne, orange, rapeseed, rhododendron, rosemary, taraxacum, tilia, sunflower, heather, buckwheat, soy, tupelo, clove, fireweed, acacia	7, 9, 26, 29-31
Isorhamnetin	0.15-4.7	Australian Jelly Bush, Manuka, Linen vine, morning, glory, black mangrove, singing bean, Christmas vine, Spanish multi-floral, rosemary, Melon, pumpkin, cherry, dandelion, maple, pinetree	7-8, 26, 28, 31

Table1-1. contd

Phytochemicals	Total Concentration range (µg/g)	Type of honeys	Reference
Acacetin	0.1-1.3	Canola, cherry, lavender, linden, lucerne, orange, rapeseed, rhododendron, rosemary, taraxacum, tilia, sunflower	30
Galangin	0.05-3.99	Canola, cherry, eucalyptus, lavender, linden, lucerne, orange, rapeseed, rhododendron, rosemary, taraxacum, tilia, sunflower, Melon, pumpkin, cherry, dandelion, maple, pinetree, heather, buckwheat, soy, tupelo, clove, acacia	7-9, 29-31
Isosakuranetin	1.30-6.20	Canola, cherry, linden, lucerne, orange, rapeseed, rosemary, taraxacum, tilia	30
Tectochrysin	0.04-2.6	Australian Jelly Bush, Canola, cherry, lucerne, orange, rapeseed, rosemary, taraxacum, tilia, Spanish multi-floral	7, 26, 30-31
Phloroglucinol	0.15-0.23	Linen vine, morning, glory, black mangrove	28
genkwanin	0.07-0.39	Spanish Multi-floral	7
rutin	1.6-5.0	Linden, heather	1
Quercetin 3-methyl ether	0.07-3.7	Australian Jelly Bush, Canola, cherry, eucalyptus, lavender, linden, lucerne, orange, rapeseed, rhododendron, rosemary, taraxacum, tilia, sunflower, Spanish multi-floral	7, 26, 30
Kaempferol 8-methyl ether	0.2-2.6	Australian Jelly Bush Manuka, Spanish multi-floral	7, 26
Quercetin 3,3' dimethyl ether	0.09-2.1	Australian Jelly Bush, Manuka, Spanish multi-floral	7, 26
8-methoxykaempferol	0.04-1.16	Linen vine, morning, glory, black mangrove, singing bean, Christmas vine, Spanish multi-floral, rosemary	7, 28, 31
2-*trans*, 4-*trans* Abscisic acid	6.3-310	Australian Jelly Bush, Manuka, Tea tree, crow ash, brush box, heath, sunflower	26, 29, 32
2-*cis*, 4-*trans* Abscisic Acid	0.2-121	Australian Jelly Bush, Manuka, buckwheat, heather, Tea tree, crow ash, brush box, heath, sunflower, soy, tupelo, clover, fireweed, acacia, Kanuka	9, 26-27, 29, 32

Table1-2. Previously reported concentrations of α-dicarbonyls in various monofloral honeys.

Honeys	Methylglyoxal	Glyoxal	3-deoxyglucosone	Reference
Manuka	Trace-1541	0.7-7.0	563-1060	17-18, 27
Multi-floral	ND-33	ND-4.6	119-1451	18, 33-34
Kanuka	0.01-174	N/A	N/A	27
Clover	1.6-16	N/A	N/A	17
Honeydew	3.7	N/A	N/A	17

N/A, not available. ND, not detected.

Table1-3. Previously reported antimicrobial activity of different varietal honeys.

Bacteria	Type of bacteria	Honey with anti-microbial activities	Possible anti-microbial compounds	Reference
Staphylococcus spp.	Infection	Coconut, gelam, lavender, heather, Manuka	gallic, caffeic, ferulic, benzoic, cinnamic, protocatequic, hydroxybenzoic acid, chlorogenic, coumaric, ellagic, naringenin, kaempferol, pinocembrin, chrysin, phenyllactic, methyl syringate, pinobanksin, pinocembrin, galangin, methylglyoxal, glyoxal, 3-deoxyglucosone	5, 18, 20, 35
E. Coli	Food safety	Coconut, gelam, lavender, heather, alfalfa, dandelion, canola, clover, buckwheat, sunflower	gallic, caffeic, ferulic, benzoic, cinnamic, protocatequic, hydroxybenzoic acid, chlorogenic, coumaric, ellagic, naringenin, kaempferol, pinocembrin, chrysin, Maillard products, methylglyoxal, glyoxal, 3-deoxyglucosone	5, 18, 35-36
Salmonella spp.	Food safety	Trefoil	pinocembrin, pinobanksin, chrysin, phenolic acids	25
Streptococcus spp	Infection	Trefoil, Manuka	pinocembrin, pinobanksin, chrysin, phenolic acids	25, 37
Bacillus spp	Food safety	Lavender, heather, Manuka, alfalfa, dandelion, canola, clover, buckwheat, sunflower	protocatequic, hydroxybenzoic acid, chlorogenic, coumaric, ellagic, naringenin, kaempferol, pinocembrin, chrysin, Maillard products	35-36

CHAPTER 2
DETERMINATION OF ANTIOXIDANT CAPACITIES, α-DICARBONYLS, AND PHENOLIC PHYTOCHEMICALS IN FLORIDA VARIETAL HONEYS USING HPLC-DAD-ESI-MS^N

Background

Honey is a natural product produced by bees from the nectar of various plants. The composition of honey is known to vary depending on the season, geographical location and the floral source. Honey is mostly composed of fructose and glucose but it has also been shown to contain phenolic phytochemicals. These compounds are mostly phenolic acid derivatives and flavonoid aglycones although some flavonoid glycosides have also been identified.[7, 28] Flavonoids are pigments in plants and they function as antioxidants, radical scavengers, antimuntagenic, anti-inflammatory and anticarcinogens.[9] Phenolic compounds, specifically flavonoids, are known to contribute to the sensory characteristics of honey, such as color, flavor and taste as well as potential health-promoting properties. The composition of these compounds was proposed as a quicker and easier way than pollen analysis to determine the floral source of honeys.[6, 26, 28, 30, 32, 38]

Honeys have also been shown to contain α-dicarbonyls including glyoxal, methylglyoxal and 3-deoxyglucosone. These compounds react with proteins to produce advanced glycation end products (AGEs) through the Maillard reaction.[33, 39] Manuka honey from New Zealand and Australia have well-documented antimicrobial properties.[16, 18, 20] The high concentration of methylglyoxal in the Manuka honeys is considered as the major factor contributing to their high antimicrobial capacity.[16, 18, 22] Manuka honeys are classified and sold according to their methylglyoxal concentration or "Unique Manuka Factor"; the larger the Unique Manuka Factor value the higher the

antimicrobial capacity. In addition to α-dicarbonyls, some phenolic compounds such as, kaempferol, quercetin and myricetin, also possess antimicrobial properties.[17, 20]

There are several studies on the phenolic composition of honey from Europe, New Zealand and Asia, but little exists on the phenolic and α-dicarbonyl composition of North American honey.[5, 11, 27] The present work aims to identify and quantify the phenolic compounds from various Florida honeys while determining their antioxidant capacities. Furthermore the identification and quantification of the main α-dicarbonyls in honey was also performed.

Materials and Methods

Chemicals

AAPH (2,2'-azotis(2-amidinopropane)) was a product of Wako Chemicals Inc. (Bellwood, RI). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), glyoxal (40% aqueous solution), pinocembrin, ellagic acid, luteolin, apigenin, morin and galangin were purchased from Sigma-Aldrich (St. Louis, MO). The 3-deoxyglucosone standard was a product of Toronto Research Chemicals Inc. (Toronto, Canada). Folin-Ciocalteu reagent, HPLC grade methanol, formic acid, acetic acid, 2-*cis*, 4-*trans*-abscisic acid, coumaric acid, rutin, quercetin, chrysin and kaempferol were purchased from Fisher Scientific (Pittsburg, PA). The C_{18} (3 ml) solid phase extraction cartridges were from Dionex (Sunnyvale, CA). Methylgloxal (40% aqueous solution) was purchased from MP Biomedicals, LLC (Solon, OH).

Samples

A total of 26 monofloral honeys were collected from Florida apiarists. These samples were classified based on the information giving by the beekeepers. The

monofloral honeys included those from orange blossom, mangrove, tupelo, palmetto, Brazilian pepper, blueberry, blackberry, gallberry, avocado, and white clover. Their harvesting region and time are listed in Table 2-1. Three Manuka honeys with certified antimicrobial capacity index 12+, 15+, and 16+ were purchased from New Zealand. Lower index number corresponds to a lower capacity while higher number corresponds to stronger antimicrobial capacity. The highest certified value for Manuka honey is 20+. A total of 17 multi-floral honeys were collected and their harvesting regions and times are listed in Table 2-2. The honeys were stored in darkness at 4°C prior to analysis.

Folin-Ciocalteu Assay

Total phenolic content was determined using the Folin-Ciocalteu assay.[40-41] Honeys (5 g) were mixed with deionized water (25 ml) and sonicated for 5 min. Each honey was then diluted with Folin-Ciocalteu reagent and 15% (w/v) sodium carbonate solution. Absorbance at 765 nm was measured on a SPECTRAmax 190 microplate reader (Molecular Devices, Sunnyvale, CA) after 30 min of incubation at room temperature. Gallic acid, 0-600 mg/L, was used to generate a standard curve. Total phenolic content was determined using three replications for each of the forty-six samples. Total phenolic content were expressed as microgram gallic acid equivalent per gram of honey (μg GAE/g).

DPPH Assay

The DPPH scavenging activities of honeys were measured according to a published method.[42] Twenty mg of DPPH were dissolved into 100 ml of methanol to make DPPH stock solution. DPPH working solution was freshly prepared by mixing 3.5 ml DPPH stock solution and 6.5 ml methanol. Absorbance at 515 nm was measured on a SPECTRAmax 190 microplate reader (Molecular Devices, Sunnyvale, CA). The initial

absorbance of DPPH working solution was between 0.9-1.0. Honeys (5 g) were mixed with 20 ml methanol and sonicated for 5 min. The honey solutions (50 µl) were added to 950 µl DPPH working solution and incubated in darkness for 60 min at room temperature in triplicates. Trolox solutions (50 µl) from 0-1,000 µM were added to DPPH working solution to produce a standard curve. DPPH scavenging activities were expressed as µmol Trolox equivalent per gram of honey (µmol TE/g).

Oxygen Radical Absorbance Capacity (ORAC) Assay

The ORAC values for each honey were determined using a modified method from Huang et al.[3] Honeys (1 g) were mixed with 10 ml phosphate buffer. The honey solutions were further diluted in phosphate buffer by 300 fold. The diluted solutions were then incubated with fluorescein as a free radical probe and AAPH as a free radical generator in duplicates. The kinetics of fluorescein degradation was read on a Spectra XMS Gemini microplate reader (Molecular Devices, Sunnyvale, CA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used to generate a standard curve (0-25µM). Antioxidant capacities were expressed as µmol Trolox equivalent per gram of honey (µmol TE/g).

Colorimeter Assay

The color of honeys was determined using a published method on a SPECTRAmax 190 microplate reader (Molecular Devices, Sunnyvale, CA).[43] Honey was diluted 50% (w/v) with warm water. The solutions were sonicated for 5 min and filtered through 0.45 µm filter units to remove large particles. The absorbance was measured at 450 nm using 720 nm as a reference wavelength to determine the color of honeys according to Beretta et al.[43]

Derivatization of α-Dicarbonyls

Alpha-dicarbonyls were analyzed as their corresponding quinoxalines after derivatization with *o*-phenylenediamine following the method from Mavric et al.[18] One ml of 15% (w/v) honeys in phosphate buffer (0.5 M, 6.5 pH) was mixed with 0.6 ml 1% (w/v) *o*-phenylenediamine in phosphate buffer. The honey solutions were kept in darkness at room temperature for at least 12 h for the reaction to complete. After derivatiztion, solutions were filtered through membrane (0.45 μm) and 20μL was injected for HPLC analysis.

HPLC Analysis of α-Dicarbonyls

An Agilent 1200 HPLC system consisting of an autosampler, a binary pump, and a diode array detector (Agilent Technologies, Palo Alto, CA) were interfaced to a HCT ion trap mass spectrometer (Bruker Daltonics, Billerica, MA). A Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm particle size, Agilent Technologies, Palo Alto, CA) was used for the separation. The binary mobile phase consisted of (A) methanol: acetic acid (99.85:0.15 v/v) and (B) methanol: acetic acid (99.97:0.03 v/v). The 49-min gradient is described as follows: 0-2 min, 20% B isocratic; 2-22 min, 20%-40% B linear; 22-37 min, 40%-100% B linear; 37-42 min, 100% B isocratic; 42-49 min 100%-20% B linear followed by 5 min of re-equilibration of the column before the next sample run.[18] The detection wavelength was 312 nm. Electrospray ionization in positive mode was performed using nebulizer 50 psi, drying gas 10 L/min, drying temperature 290 $^{\circ}$C, and capillary of 4000 V. The full scan mass spectra of the quinoxalines were recorded in a range of *m/z* 100-3000. Auto MS^2 was conducted with 80% compound stability and 80% trap drive level. Pure compounds of methylglyoxal, glyoxal and 3-deoxyglucosone were used as external standards to quantify the quinoxalines formed after derivatization.

The concentration range of external standard for 3-deoxglucosone, glyoxal, and methylglyoxal were 3-520 mg/L, 0.1-60 mg/L, and 0.1-250 mg/L, respectively. The α-dicarbonyls were identified on the basis of full scan and product ion mass spectra, UV/Vis spectra on diode array detector and compared to authentic standards.[18, 33-34]

Solid Phase Extraction of Phenolic Phytochemicals

The procedure for the extraction of phenolic phytochemicals from honeys was adapted from Hadjmohammadi et al.[3] Honey (10 g) was mixed with 40 ml deionized water. The solution was filtered to remove any solid residue. The SPE C18 cartridges (0.5 g packing) were preconditioned with 6 ml of methanol followed by 3 ml of deionized water. The honey solutions were loaded on the individual cartridges and rinsed with 15 ml of water and 3 ml of 10% (v/v) methanol to remove sugars. The phenolic fractions were recovered by eluting the cartridge with 6 ml methanol. The eluent was dried in a SpeedVac concentrator (Thermo scientific ISS110, Waltham, MA) overnight at room temperature. The dried extracts were then dissolved in 500 µl methanol, sonicated for 10 min, and centrifuged at 13,300 rpm for 5 min. Twenty microliters of the supernatant was injected for analysis on HPLC.

HPLC Analysis of Phenolic Phytochemicals

A Zorbax SB-C18 column (250 mm × 4.6 mm, 5 µm particle size, Agilent Technologies, Palo Alto, CA) was used for the separation. The binary mobile phase consisted of (A) water:formic acid (99.5:0.5, v/v) and (B) methanol at a constant flow rate of 0.8 ml. A 60 min gradient was adapted from a published paper.[8, 44] The gradient is described as follows: 0-5 min, 5% B isocratic, 5-20 min, 5%-30% B linear, 20-32 min, 30%-35% B linear, 32-45 min, 35%-50% B linear, 45- 50 min, 50%-80% B linear, 50-55 min, 80%-5% B linear followed by 5 min of re-equilibration of the column before the next

injection. The detection wavelengths were set at 290 nm, 340 nm and 520 nm, since a majority of the phenolics' UV absorption maxima are at these wavelengths.[26, 45-47] Electrospray ionization in negative mode was performed using nebulizer 50 psi, drying gas 11 L/min, drying temperature 350 ^{o}C, and capillary of 4000 V. The full scan mass spectra of the phytochemicals were recorded from *m/z* 100 to 1000. Auto MS^2 was conducted with 80% compound stability and 50% trap drive level. Galangin, pinocembrin, quercetin, 2-*cis*, 4-*trans*-abscisic acid, luteolin, rutin, ellagic acid, coumaric acid, chrysin, apigenin and kaempferol were used as external standards to quantify flavonoids and phenolic acids. Phenolic phytochemicals were identified using a combination of full scan and product ion mass spectra, UV/Vis spectra on diode array detector with comparison to authentic standards, and comparison with published papers.[9]

Statistical Analysis

Data was expressed as mean ± standard deviation of three independent observations per each honey sample unless otherwise stated. Total phenolic content, antioxidant capacities, and color of honeys were grouped into four quartiles. The lowest and highest values were in quartiles 1-25th and 75-100th, respectively. Pearson correlation for the different comparable assays was determined using SigmaPlot software (Version 12, Systat software Inc., Chicago, IL). Hierarchical clustering analyses of honeys were performed using JMP software (Version 9.0, SAS institute Inc. Cary, NC). These clusters determined the similarity of honey varieties based on the phenolic phytochemical concentration of each honey sample.

Results and Discussion

The phytochemical constituents and concentration of honeys can differ greatly depending on the floral source. The presence of flavonoid and phenolic derivatives has been seen in propolis but not extensively in honey. Another unique feature is that different varieties of honey possess α-dicarbonyls that contribute to honeys antimicrobial capacity. Color can also help to indicate the extent of both the α-dicarbonyl and phenolic compound concentrations.

Total Phenolic Content, Antioxidant Capacity, and Color Analysis

Table 2-3 shows the total phenolic content, antioxidant capacity, and color analysis results of 26 monofloral honeys analyzed, including three New Zealand Manuka honeys. Results for 17 multi-floral honeys are shown in Table 2-4. The total phenolic content in the monofloral and multi-floral honeys ranged from 250 to 1,570 μg GAE/g on the basis of fresh weight. This range of values was in agreement with previously published values of honeys from other regions of the world.[9-11, 14, 43] The two individual avocado honeys examined had the highest total phenolic content (averaged 1,450 μg GAE/g) among all the honeys. This value is similar to those reported in honeydew honeys (1,150 μg GAE/g) from Burkina Faso.[10] The highest total phenolic content in the multi-floral honeys was 1,040 μg GAE/g in sample 14. This honey was collected from southern Florida during the fall season. There was no apparent correlation between total phenolic content and harvesting time or location for the multi-floral honeys. This may be due to the fact that Florida's subtropical climate allows for the same plants to be grown in multiple seasons and multiple areas of the state. A previous study from Cuba and Portugal showed that the highest total phenolic content for honeys produced in these countries were 700 μg GAE/g.[29, 35] These contents were

comparable to Florida monofloral honeys of mangrove, tupelo, and palmetto, which had average total phenolic contents of 730, 651, and 786 µg GAE/g, respectively. The multi-floral varieties also had a similar average total phenolic content of 748 µg GAE/g.

It has been shown that color can be an indicator of total phenolic content and honeys antioxidant capacity. Darker honeys have often shown a higher phenolic content and antioxidant capacity.[11, 43] The color of honeys ranged from 55 mAU in a light colored white clover honey to 1,764 mAU in a dark avocado honey. The range of absorbance is consistent with previously reported values.[36, 43, 48] Orange blossom honeys 2b and 2d and white clover honey 11b had the lowest absorbance and were visually whiter than the other varieties. The two avocado honeys, 10a and 10b, had the highest absorbance at 604 mAU and 1,764 mAU, respectively, among all the honeys analyzed. These two honeys also showed the highest total phenolic content among all the honeys examined. Multi-floral honey 24 showed the lowest values for color, total phenolic content and antioxidant capacity (Table 2-4). These observations supported the conclusion that color and total phenolic content in honey are directly correlated. The multi-floral variety had an average absorbance of 305 mAU, which was similar to the average absorbance of monofloral honeys (290 mAU). The color of honeys can be attributed to flavonoids, other plant pigments and Maillard reaction products. Floral source, processing and storage conditions of honeys may affect pigment content in honey and cause variation in absorbance. The correlation coefficient (*r*) between honeys total phenolic content and color was 0.669 with a p-value of <0.05. This shows there was a significant correlation between color and total phenolic content.

The DPPH free radical scavenging activity of monofloral honeys ranged from 0.282 to 3.33 μmol TE/g honey. Avocado honey, 10a and 10b, had the highest free radical scavenging capability among all honeys. The orange blossom honeys 2b and 2d and the white clover honey 11b had the lowest free radical scavenging values at 0.282, 0.384 and 0.440 μmol TE/g honey, respectively. Honey with the highest radical scavenging values (i.e., avocado honey) had the darkest color as indicated by their high absorbance values. Honeys with paler colors, such as orange blossom and white clover honeys had lower absorbance and scavenging capacities compared to other varieties. It has been suggested that darker honeys generally have higher radical scavenging potential.[43] The free radical scavenging capacity of multi-floral varieties ranged between 0.875-2.19 μmol TE/g. The correlation coefficient (*r*) between total phenolic concentration and DPPH concentration was 0.596 with a p-value <0.05. This r-value is lower than those reported between total phenolics and free radical scavenging in other foods.[10, 43] This difference could be attributed to different storage or processing methods performed by the apiarists and varying sugar concentrations in honeys.[34]

The antioxidant capacity of honeys, expressed as ORAC values, ranged from 1.48 μmol TE/g to 28.0 μmol TE/g honey, which are comparable with those reported previously.[43, 49] The ORAC value of tupelo honey 4c was significantly higher than all other honeys tested. The average ORAC value for the four other different tupelo honeys, 4a, 4b, 4d and 4e, was 13.4 μmol TE/g honey, which was much lower than tupelo honey 4c of 28.0 μmol TE/g. Tupelo honey 4c also had the highest total phenolic content and DPPH values out of all tupelo honeys. A possible explanation of high phenolic content in honey 4c was that this honey was from different species of Tupelo,

as multiple varieties can be found in Florida.[50] The multi-floral varieties had an average ORAC value of 9 μmol TE/g, which is comparable to the Brazilain pepper honeys and the gallberry monofloral honeys (10 μmol TE/g). The correlation coefficient (*r*) between total phenolic concentration of the honeys and ORAC values was 0.577 with a p-value<0.05. The ORAC assay does not measure antioxidant capacity due to phenolics alone, amino acids and other nucleophiles may also quench the free radical AAPH in this method.

Identification and Quantification of α-Dicarbonyls

Figure 2-1A shows the HPLC-DAD chromatogram of the standard mixture of, 3-deoxyglucosone, glyoxal, and methylglyoxal. These compounds were detected using UV/Vis detection at 312 nm after a derivatization step with *o*-phenylenediamine to form their respective quinoxalines. The retention time, $[M+H]^+$, and product ions of identified quinoxalines are listed in Table 2-5. Peak 1 was identified as excessive *o*-phenlyenediamine. Peak 2 was tentatively identified as glucosone according to $[M+H]^+$ 251 and a published study.[34, 51-52] Peak 3 produced *m/z* 235 $[M+H]^+$, which fragmented into *m/z* 217 and 199. These spectra were consistent with 3-deoxyglucosone.[34, 52] Peak 4 was identified as glyoxal by comparing UV/Vis and retention time with standard. Peak 5 yielded *m/z* 145 $[M+H]^+$ and was identified as the quinoxaline formed after the reaction between *o*-phenylenediamine and methylglyoxal.[18, 34] The profile of dicarbonyls in all honeys was shown to be similar (Figure 2-1B and 2-1C). However, the concentration of these carbonyls varied depending on the floral source examined. For example, Manuka honey (Figure 2-1B) showed a predominant peak for methylglyoxal whereas methylglyoxal concentration in all of the other varieties was much lower (Table 2-6 and Table 2-7). Figure 2-2A, 2-2B, 2-2C and 2-2D show the

chromatograms of α-dicarbonyls in avocado honey 10a, orange blossom honey 2a, palmetto honey 5b and tupelo honey 4b, respectively. Figure 2-3 depicts the α-dicarbonyl profiles in multi-floral varieties, 14, 23, and 25. Results demonstrated that α-dicarbonyl profile in honeys differ according to floral sources and geographical locations.

Table 2-6 and Table 2-7 provide the concentrations of glyoxal, methylglyoxal, and 3-deoxyglucosone in monofloral and multi-floral honeys, respectively. Glucosone was not quantified in this experiment due to its artificial formation in select buffers and sample matrixes. Samples that contain metal ions in the sample matrix can slow the reaction rate of glucosone with *o*-phenylenediamine and make it difficult to accurately quantify.[51] The content of methylglyoxal in the three Manuka honeys 1a, 1b and 1c were 86.9, 483, and 92.1 μg/g, respectively. Methylglyoxal concentration in other monofloral honeys ranged from 3.45 to 13.0 μg/g. Methylglyoxal in Manuka was 7-37 times more concentrated than the next highest monofloral honey 9c. Other studies have seen methylglyoxal concentrations ranging from undetectable to over 1,500 μg/g in some Manuka varieties.[16, 18, 22-23, 27] Adams et al. reported methylglyoxal concentrations in Manuka honeys from 25-709 μg/g and 1.6-24 μg/g in other varieties using a similar derivatization method.[17] Stephen et al. showed that the longer a Manuka honey was aged the higher the methylglyoxal concentration became.[27] The high levels of methylglyoxal in the Manuka honeys have not been seen in any other foods.[18] A number of researchers suggested that the high levels of methylglyoxal in Manuka honeys is the major contributing factor to its high antimicrobial capacity.[18] Glyoxal concentrations ranged from 1.78-7.35 μg/g, which is similar to the concentrations

reported in honeys collected from other regions of the world.[16-18, 36] Among the three α-dicarbonyls quantified, 3-deoxyglucosone had the highest concentration in all honeys. The higher concentration of 3-deoxyglucosone than methylglyoxal and glyoxal in honeys is expected because 3-deoxyglucosone is the initial product of glucose degradation and honey has a high sugar content.[33] Retroaldolization of 3-deoxyglucosone produces methylglyoxal.[33] Thus, when 3-deoxyglucosone decreases, methylglyoxal should increase. This was not confirmed as a processing study was not performed on honeys to examine this reaction mechanism.

Identification and Quantification of Phenolic Phytochemicals

Figures 2-6, 2-7, 2-8, and 2-9 depict the HPLC chromatograms of phenolic phytochemicals in different honeys after samples were purified using solid phase extraction. The chromatograms were recorded at 290, 340 and 520 nm, although only the chromatograms at 290 nm are shown. This is because most phenolic compounds can be detected at this wavelength.[41] Mass spectrometry was operated in the negative mode due to its higher sensitivity towards flavonoids than positive ionization mode.[53] Phenolic compounds in honeys were identified based on a combination of mass spectra, UV/Vis spectra from diode array detector, and comparison with standards. The retention time, $[M-H]^-$, and production ions of identified phenolic acids and flavonoids are listed in Table 2-8. Nine compounds were identified by comparing with authentic standards and using MS^n. Additionally three compounds were tentatively identified using MS^n. These compounds included flavones, flavonols, flavanones, isomers of the terpenoid abscisic acid, phenolic acids, and phenolic derivatives. These compounds had all been previously identified in other honey varieties or propolis.[6, 26, 32, 45, 54-60] Figure 2-4A through 2-4C display the mass spectra of the three compounds that were

tentatively identified. No compounds were identified from honeys 6c and 27 due to poor separation of peaks.

Phenolic Acids: Peaks 1 was confirmed to be coumaric acid by comparing retention time and mass spectra with authentic standards.

Terpenoids: Peak 4 was identified as 2-*cis, 4-trans*-abscisic acid by comparing with authentic standard. It produced an $[M-H]^-$ at *m/z* 263 that further dissociated to form *m/z* 219 and *m/z* 153 after loss of CO_2 and the side chain, respectively. Peak 3 also produced *m/z* 263 $[M-H]^-$ (Figure 2-4A). This peak had two main fragments *m/z* 204 after the loss of both CO_2 and CH_3 and *m/z* 219 after the loss of CO_2. The peak was tentatively identified as 2-*trans, 4-trans*-abscisic acid. Fragmentation pattern of 2-*trans, 4-trans*-abscisic acid are shown in Figure 2-5A. Both isomers of abscisic acid had been identified in other honey varieties.[26, 29, 57, 59]

Flavanones: Peak 10 was identified as pinocembrin by comparing retention time and mass spectra with its standard. Peak 10 gave an ion at *m/z* 255 $[M-H]^-$, which further dissociated into *m/z* 213 from the loss of C_2H_2O [42Da] and *m/z* 151 from the RDA reaction.[56]

Flavonols: Multiple flavonols were identified in the honeys. Peak 2 was rutin. It produced *m/z* 609 $[M-H]^-$, which further dissociated into *m/z* 301 after the loss of the rutinose group corresponding to the deprotonated quercetin aglycone.[53, 61] Peak 6 was confirmed to be quercetin. The maximum absorbance was seen at 340nm. Its retention times and mass spectrum matched those of an authentic standard. Peak 9 was identified as kaempferol by comparing retention time and mass spectra with authentic standards. Kaempferol produced an ion at *m/z* 285 $[M-H]^-$ and two product ions at *m/z*

267 [M-H-H_2O]$^-$ and *m/z* 151 from RDA reaction. Peak 10 contained two flavonoids, galangin and chrysin. Galangin produced an ion with *m/z* 269 [M-H]$^-$, which further dissociated into *m/z* 197, which was formed from the loss of both a CO_2 and a CO [62 Da] group and *m/z* 227.

Flavanonols: The product ion spectrum of peak 5 is depicted in Figure 2-4B. This peak produced *m/z* 285 [M-H]$^-$, which further dissociated into multiple ions *m/z* 267 from the loss of water and *m/z* 252 from the combine loss of methyl and water (Figure 2-5B). It was tentatively identified as pinobanksin-5-methyl-ether by comparing the mass spectra with previous research.[54-55] Peak 7 produced *m/z* 271 [M-H]$^-$, which further dissociated into a main fragment *m/z* 253 corresponding to the loss of water (Figure 2-4C). Its fragmentation pattern can be seen in Figure 2-5C and was tentatively identified as pinobanksin.[55] Pinobanksin and its derivatives had been previously identified in honey and other bee products.[54-55]

Flavones: Luteolin (peak 8) and chrysin (peak 11) were identified in honey by comparing retention time and mass spectra with standards. These flavones had been previously identified in many different varieties of honeys and thus their presence would not be beneficial in authenticating a particular variety of honey.[31, 38, 62]

The concentrations of the 11 phenolic compounds quantified in monofloral and multi-floral honeys are listed in Table 2-9 and Table 2-10, respectively. HPLC chromatograms showed that phenolic phytochemical in palmetto honeys 5a (Figure 2-6A) and 5b (Figure 2-6B) had similar profiles. These profiles show the phenolic “finger prints” of honeys and my help to identify floral source of unknown honeys. It was speculated that the multi-floral honeys could be classified according to composition of

phenolic phytochemicals. The Manuka honeys 1a (Figure 2-7A) and 1b (Figure 2-7B) had two large peaks that were unidentifiable from mass data. Honey is a complex mixture that may include other compounds such as pesticides, antibiotics, peptides, amino acids, dicarboxylic acids and other residues that may not be easily identifiable with the current method.[63-66] Figure 2-8A, 2-8B, and 2-8C represent the chromatograms of phenolic phytochemicals in orange blossom honey 2a, avocado honey 10a, and gallberry honey 9b, respectively. The avocado honey was the only sample in which neither pinocembrin or chrysin were detected.

The concentration and profiles of phenolics in the multi-floral honeys varied considerably according to seasons, locations, and apiarists. These differences can be seen in Figure 2-9A and 2-9B that show phenolic composition in multi-floral honeys, 13, and 23, respectively. There was a higher concentration of abscisic acids in honey 13 than honey 21. Both honeys were form central Florida but 13 was collected in the summer and 23 was collected in the fall.

A majority of the phenolic compounds identified and quantified were in small amounts less than 1 μg/g. The plant hormone, abscisic acid was seen in honeys in two isomeric forms 2-*cis, 4-trans*-abscisic acid and 2-*trans, 4-trans*-abscisic acid. Both forms were seen in all honeys except the trans-trans form was not seen in honey 1b and 20. 2-*cis, 4-trans*-abscisic acid (peak 5) was the most abundant phytochemical found in most of the honeys. The presence of abscisic acid was not surprising as it is commonly seen in all plants. Abscisic acid has also been documented in other honey varieties.[26, 29, 57, 59]

Hierarchical Cluster Analysis

Monofloral honeys were clustered according to averaged phenolic concentration. The orange blossom and palmetto honeys appeared to be most similar (Figure 2-10). Brazilian pepper and Manuka honeys were also similar as well as the mangrove and avocado honeys. For multi-floral honeys, samples 12 and 21 appeared to be very similar (Figure 2-11). Both samples were harvested from central Florida. Honey 16 did not cluster well with the other honeys. This honey had high levels of 2-*trans*, 4-*trans*-abscisic acid and 2-*cis*, 4-*trans*-abscisic acid.

Honey samples in this study were collected by experienced beekeepers. Floral source of honey were reported by beekeepers but were not verified by pollen counting. It is often difficult to determine the floral sources of a honey because bees can forage on over 32,000 acres. We speculated that some of monofloral honeys may contain honeys from other floral sources. This remains a limitation of this study.

Summary

Our results indicate that Florida honeys have similar phenolic content and antioxidant capacities to other honeys studied. The higher antioxidant capacity and total phenolic content of the avocado variety makes them potentially a better honey when evaluating health-promoting properties. Additionally, it was confirmed from the results that HPLC – ESI – MS^n is a valuable tool for the identification of phenolic compounds in honeys. This method was useful in extracting and identifying the prominent flavonoid compounds in honeys. The prominent compounds in Florida honeys were the isomers of the plant hormone abscisic acid.

Table2-1. Floral sources, harvesting region and time of monofloral honeys.

Sample No.	Floral Type	Region of Florida	Season Harvested	Sample No.	Floral Type	Region of Florida	Season Harvested
1a	Manuka 16+	New Zealand	Unknown	5c	Palmetto	South	Unknown
1b	Manuka 15+	New Zealand	Unknown	5d	Palmetto	South	Unknown
1c	Manuka 12+	New Zealand	Unknown	6a	Brazilian Pepper	Central	Fall 2010
2a	Orange blossom	Central	Spring 2011	6b	Brazilian Pepper	Central	Unknown
2b	Orange blossom	North	Unknown	6c	Brazilian Pepper	North	Fall 2010
2c	Orange blossom	South	Unknown	7	Blueberry	South	Unknown
2d	Orange blossom	Central	Unknown	8	Blackberry	North	Summer 2010
3	Mangrove	South	Unknown	9a	Gallberry	Central	Unknown
4a	Tupelo	Central	Unknown	9b	Gallberry	North	Unknown
4b	Tupelo	North	Unknown	9c	Gallberry	Central	Unknown
4c	Tupelo	North	Unknown	10a	Avocado	Central	Unknown
4d	Tupelo	North	Unknown	10b	Avocado	South	Unknown
4e	Tupelo	North	Unknown	11a	White Clover	South	Unknown
5a	Palmetto	North	Spring 2010	11b	White Clover	North	Unknown
5b	Palmetto	Central	Summer 2010				

Table2-2. Harvesting region and time of multi-floral honeys.

Sample No.	Floral Type	Region of Florida	Season Harvested
12	Wild	Central	Fall 2010
13	Wild	North	Summer 2010
14	Wild	South	Fall 2010
15	Wild	Central	Fall 2010
16	Wild	Central	Fall 2010
17	Wild	Central	Summer 2010
18	Wild	North	Fall 2010
19	Wild	Central	Fall 2010
20	Wild	Central	Fall 2010
21	Wild	Central	Summer 2010
22	Wild	North	Spring 2011
23	Wild	Central	Fall 2010
24	Wild	Central	Fall 2010
25	Wild	Central	Spring 2011
26	Wild	Central	Fall 2010
27	Wild	Central	Fall 2010
28	Wild	North	Fall 2010

Table2-3. Total phenolic content, free radical scavenging, antioxidant capacities, and color analysis of monofloral honeys.

Honeys	Total Phenolics (µg/g)	DPPH (µmol TE/g honey)	ORAC (µmol TE/g honey)[#]	ABS_{450} (AU, 50% w/v)
1a	1,080±14.2[a]	1.37±0.665[b]	15.4±9.31[a]	0.298±0.018[b]
1b	774±27.0[b]	1.35±0.218[b]	6.92±0.233	0.310±0.014[a]
1c	1,030±46.1[a]	1.38±0.244[b]	11.3±3.22[b]	0.224±0.010[c]
2a	829±17.8[b]	1.40±0.005[b]	6.16±3.35[c]	0.348±0.018[a]
2b	593±28.4[c]	0.282±0.115[d]	1.48±1.36[d]	0.077±0.005[d]
2c	386±14.5[d]	0.686±0.262[d]	4.26±0.961[d]	0.117±0.013[d]
2d	286±3.30[d]	0.384±0.251[d]	4.87±2.08[d]	0.084±0.003[d]
3	730±33.9[c]	2.16±0.172[a]	7.46±0.329[b]	0.489±0.040[a]
4a	820±23.0[b]	1.36±0.324[b]	7.69±0.639[b]	0.241±0.017
4b	773±15.7[b]	1.13±0.182[c]	6.86±1.14[c]	0.145±0.003[d]
4c	997±12.4[a]	1.94±0.148[a]	28.0±1.40[a]	0.218±0.012[c]
4d	649±25.8[c]	1.77±0.090[a]	11.8±1.07[b]	0.208±0.011[c]
4e	691±15.8[c]	1.78±0.099[a]	12.7±4.56[b]	0.192±0.010[c]
5a	767±27.1	0.679±0.231[d]	5.09±0.420[c]	0.163±0.008[c]
5b	851±12.3[b]	1.22±0.250[c]	12.8±0.961[a]	0.248±0.065[b]
5c	530±7.76[d]	1.22±0.130	4.77±0.632[d]	0.284±0.065[b]
5d	457±1.73[d]	0.938±0.137[c]	5.77±0.486[c]	0.143±0.008[d]
6a	919±11.2[a]	1.66±0.018[b]	5.48±3.38[c]	0.353±0.021[a]
6b	891±23.1[b]	1.55±0.079[b]	15.0±3.85[a]	0.264±0.012[b]
6c	643±13.3[c]	2.18±0.183[a]	10.6±0.683[b]	0.292±0.017[b]
7	466±4.91[d]	0.723±0.287[d]	3.13±3.98[d]	0.269±0.012[b]
8	532±31.1[d]	0.753±0.184[d]	6.27±0.592[c]	0.168±0.005[c]
9a	1,000±13.7[a]	0.826±0.326[c]	16.5±1.78[a]	0.272±0.010[b]
9b	694±47.6[c]	0.970±0.077[c]	3.18±0.575[d]	0.115±0.000[d]
9c	736±11.9[c]	0.870±0.128[c]	11.6±2.91[b]	0.172±0.115[c]
10a	1,360±9.93[a]	2.75±0.267[a]	15.5±6.12[a]	0.604±0.020[a]
10b	1,570±33.4[a]	3.33±0.183[a]	14.3±4.03[a]	1.76±0.065[a]

Table2-3. contd

Honeys	Total Phenolics (µg/g)	DPPH (µmol TE/g honey)	ORAC (µmol TE/g honey)[#]	ABS_{450} (AU, 50% w/v)
11a	845±41.0[b]	0.893±0.145[c]	3.85±0.06[d]	0.299±0.049[a]
11b	251±17.6[d]	0.440±0.041[d]	6.27±0.59[c]	0.055±0.002[d]
Range	251-1,570	0.282-3.33	1.48-28.0	0.055-1.76
Average	735	1.31	9.13	0.290

Results are mean ± SD of three determinations on fresh weight basis. #ORAC values are mean of two determinations. Values in each column are grouped into four quartiles (a>75th, b=50th-75th, c=25th-50th, and d<25th percentile, median not labeled). Numbers in bold are low, high and median values in each column.

Table2-4. Total Phenolic content, free radical scavenging, antioxidant capacities, and color analysis of multi-floral honeys

Honeys	Total Phenolics (µg/g)	DPPH (µmol TE/g honey)	ORAC (µmol TE/g honey)[#]	ABS_{450} (AU, 50% w/v)
12	853±25.8[b]	1.85±0.156[b]	6.62±4.79[c]	0.292±0.013
13	936±3.84[a]	1.08±0.014[d]	18.2±5.10[a]	0.220±0.017[d]
14	1,040±12.6[a]	1.49±0.233[b]	15.9±1.68[a]	0.341±0.019[b]
15	933±17.6[a]	1.66±0.111[b]	6.05±1.07[c]	0.412±0.019[a]
16	844±13.1[b]	1.10±0.217[d]	10.8±2.37[b]	0.267±0.016[c]
17	981±6.82[a]	1.28±0.132[c]	4.67±3.16[d]	0.480±0.035[a]
18	880±39.6[b]	1.48±0.220[b]	12.6±2.20[a]	0.304±0.004[b]
19	662±12.1[c]	2.07±0.124[a]	6.70±0.040[c]	0.380±0.056[a]
20	670±22.6[c]	2.07±0.05[a]	12.7±2.99[a]	0.233±0.011[d]
21	649±18.7[c]	2.02±0.319[a]	4.13±0.57[d]	0.282±0.013[c]
22	478±10.2[d]	1.27±0.113[c]	9.34±6.24[b]	0.299±0.035[b]
23	815±15.8	1.69±0.116[b]	7.21±2.58[b]	0.508±0.053[a]
24	400±5.24[d]	0.875±0.101[d]	2.20±0.087[d]	0.149±0.009[d]
25	604±14.8[d]	1.43±0.188[c]	7.05±0.632	0.301±0.018[b]
26	518±9.31[d]	1.13±0.048[d]	4.70±0.851[d]	0.242±0.008[d]
27	630±2.10[c]	2.19±0.116[a]	11.8±2.37[b]	0.251±0.014[c]
28	846±32.0[b]	1.48±0.207	5.67±6.97[c]	0.261±0.016[c]
Range	400-1,040	0.875-2.19	2.20-18.2	0.149-0.508
Average	748	1.42	8.87	0.305

Results are mean ± SD of three determinations on fresh weight basis. #ORAC values are mean of two determinations. Values are grouped into four quartiles (a>75th, b=50th-75th, c=25th-50th, and d<25th percentile, median not labeled). Numbers in bold are low, high and median values in each column.

Table2-5. Identification of α-dicarbonyls after derivatization with *o*-phenylenediamine using HPLC-ESI-MS^n.

Peak	Identified Compound	RT (min)	$[M+H]^+$ (*m/z*)	MS^2 (*m/z*)
1	o-phenylenediamine	4.9	109	none
2	glucosone	14.4	251	233, 173, 215
3	3-deoxyglucosone	20.4	235	217, 199
4	Glyoxal	31.6	ND	ND
5	Methylglyoxal	33.6	145	119

ND-not determined on MS^n.

Table2-6. α-dicarbonyl content of monofloral honeys.

Sample No.	3-Deoxyglucosone (μg/g)	Glyoxal (μg/g)	Methylglyoxal (μg/g)	Total Carbonyl (μg/g)
1a	636±48.8	4.39±0.660	86.9±0.967	727±49.8
1b	558±13.2	5.13±0.074	483±10.7	1047±23.6
1c	646±40.2	3.09±0.182	92.1±0.343	741±39.8
2a	684±34.5	4.53±1.01	6.09±1.27	695±33.2
2b	288±11.9	5.70±0.462	6.19±1.46	300±12.7
2c	463±6.13	2.96±0.127	4.24±0.396	471±5.62
2d	206±21.4	2.19±0.345	3.68±1.49	212±21.6
3	808±16.6	2.92±0.111	5.15±0.277	816±16.8
4a	614±23.4	2.90±1.19	4.85±0.619	622±24.8
4b	502±23.5	3.93±1.22	5.90±1.35	512±24.5
4c	267±10.3	6.49±0.110	7.98±0.172	281±10.3
4d	377±11.2	1.78±0.228	4.21±0.375	383±11.8
4e	259±12.7	2.68±0.257	4.97±0.582	266±13.3
5a	474±35.1	2.95±0.222	5.80±0.255	483±34.8
5b	831±3.46	6.10±0.315	10.8±1.77	848±23.2
5c	730±28.7	2.23±0.109	4.53±1.01	737±29.1
5d	612±10.8	3.63±0.292	3.88±1.49	620±9.59
6a	693±11.2	2.78±0.59	4.03±0.717	699±11.5
6b	473±2.60	4.33±0.084	6.40±1.05	484±2.68
6c	890±40.0	2.33±0.271	4.50±0.235	897±40.3
7	746±37.8	3.92±0.321	8.78±0.539	759±37.9
8	551±70.0	2.19±0.338	4.34±0.471	557±70.7
9a	517±3.15	3.75±1.39	7.09±2.52	528±6.55
9b	466±24.9	2.76±0.694	4.88±1.05	474±26.6
9c	701±39.9	7.35±0.935	13.0±4.30	722±35.3
10a	2557±64.4	4.00±0.756	6.19±0.920	2568±65.9
10b	3662±76.9	7.23±0.175	9.98±0.575	3679±77.6
11a	1209±42.0	3.31±0.362	9.58±0.883	1221±41.5
11b	282±4.95	3.57±0.333	6.23±0.212	292±5.17
Range	206-3662	1.78-7.23	3.68-483	212-3679
Average	748	4.03	28.5	781

Results are mean ± standard deviation of three determinations on fresh weight basis.

Table2-7. α-dicarbonyl content of multi-floral honeys.

Sample No.	3-Deoxyglucosone (µg/g)	Glyoxal (µg/g)	Methylglyoxal (µg/g)	Total Carbonyl (µg/g)
12	883±3.46	5.32±0.892	7.44±0.615	896±2.10
13	372±17.2	2.44±0.778	5.23±1.15	380±19.0
14	754±17.6	4.11±1.56	6.80±1.98	765±18.0
15	817±75.0	3.29±0.207	4.90±0.529	825±74.5
16	786±52.9	3.80±0.439	6.61±0515	796±53.7
17	678±109	3.96±0.915	5.31±0.948	687±111
18	459±9.05	3.22±0.087	6.98±0.388	469±9.44
19	884±21.8	2.49±0.321	3.63±0.284	890±22.3
20	519±34.0	1.63±0.151	3.45±0.194	524±34.1
21	901±15.0	3.22±0.211	6.27±0.339	911±15.3
22	727±8.95	3.27±0.245	6.38±0.187	753±37.7
23	1239±162	5.22±0.194	6.76±0.485	1251±161
24	359±25.8	2.02±0.107	4.66±0.884	366±26.8
25	555±8.74	2.69±0.333	4.76±1.53	563±8.90
26	523±8.96	3.20±0.151	6.03±1.70	533±9.23
27	662±9.82	2.89±0.159	5.20±0.248	670±9.55
28	655±13.6	3.89±0.370	6.97±0.762	666±13.4
Range	359-1239	1.63-5.32	3.45-7.44	366-1251
Average	693	3.33	5.73	703

Results are mean ± standard deviation of three determinations on fresh weight basis.

Table2-8. Identification of phytochemicals in honeys by HPLC-DAD-ESI-MS^n.

Peak No.	Compound	Molecular weight	Retention time (min)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)
1	Coumaric Acid	164	22.5	163	119
2	Rutin	610	32	609	301
3	*2-trans, 4-trans*-abscisic acid*	264	34.7	263	219, 204, 201
4	*2-cis, 4-trans*-abscisic acid	264	37.8	263	219, 153
5	Pinobanksin 5-methyl ether*	286	39	285	267, 252, 239
6	Quercetin	302	39.7	301	179, 151
7	Pinobanksin*	272	40.4	271	253, 215, 151
8	Luteolin	286	42	285	243, 223, 199
9	Kaempferol	286	42.7	285	257, 151
10	Pinocembrin	256	46.3	255	213, 151
11	Chrysin	254	48	253	209
	Galangin	270		269	227, 213, 197

Nine compounds were identified by comparing with authentic standards. Three compounds with * were tentatively identified by mass spectra.

Table2-9. Content of phenolic compounds in monofloral honeys (µg/g).

Sample	Coumaric acid	Rutin	*2-trans, 4-trans*-abscisic acid	*2-cis, 4-trans*-abscisic acid	Quercetin
1a, Manuka 16+	0.808±0.04	ND	0.710±0.11	1.55±0.19	0.552±0.06
1b, Manuka 15+	ND	ND	ND	0.525±0.22	0.495±0.24
1c, Manuka 12+	0.103±0.04	ND	1.54±0.392	2.77±0.69	0.396±0.11
2a, Orange blossom	ND	0.019±0.008	5.07±1.09	29.1±7.92	0.278±0.097
2b, Orange blossom	0.437±0.26	0.055±0.002	5.80±0.08	15.1±0.11	0.650±0.04
2c, Orange blossom	ND	0.083±0.039	0.100±0.04	0.486±0.21	0.502±0.24
2d, Orange blossom	0.380±0.12	0.060±0.001	2.03±0.11	5.12±0.27	1.15±0.06
3, Mangrove	0.287±0.03	0.036±0.005	1.27±0.28	5.36±0.74	0.350±0.12
4a, Tupelo	0.604±0.29	ND	19.1±5.01	27.0±1.94	1.224±0.07
4b, Tupelo	ND	ND	7.50±0.36	9.70±0.38	0.436±0.04
4c, Tupelo	0.356±0.09	0.118±0.013	17.1±1.87	18.2±3.15	1.70±0.48
4d, Tupelo	0.437±0.14	ND	20.5±1.15	24.0±1.71	0.646±0.32
4e, Tupelo	0.355±0.18	0.031±0.021	4.53±1.34	5.95±0.78	ND
5a, Palmetto	0.745±0.03	0.034±0.005	14.4±0.40	25.5±1.00	0.689±0.244
5b, Palmetto	ND	0.053±0.012	12.7±1.86	21.1±2.47	ND
5c, Palmetto	1.208±0.49	0.036±0.014	2.08±0.73	8.00±2.73	0.711±0.25
5d, Palmetto	0.274±0.14	0.087±0.001	0.333±0.07	0.472±0.03	0.939±0.11
6a, Brazilian Pepper	0.147±0.03	ND	0.664±0.03	4.67±0.07	ND
6b, Brazilian Pepper	0.187±0.07	0.070±0.013	0.892±0.39	2.51±0.62	0.399±0.06
7, Blueberry	1.443±0.21	0.022±0.004	1.14±0.05	3.38±0.12	0.304±0.03
8, Blackberry	0.403±0.03	0.015±0.001	1.49±0.22	3.75±0.06	0.241±0.02
9a, Gallberry	0.185±0.05	ND	7.53±0.49	24.0±1.77	0.317±0.24
9b, Gallberry	0.295±0.14	ND	26.1±11.1	27.6±11.8	0.341±0.161
9c, Gallberry	0.508±0.16	0.022±0.003	13.1±1.01	24.3±2.57	0.344±0.07
10a, Avocado	0.157±0.08	0.017±0.003	11.5±3.35	26.2±3.17	0.108±0.02
10b, Avocado	0.224±0.10	0.089±0.018	1.85±0.39	6.17±1.25	0.747±0.103
11a, White Clover	0.201±0.04	0.127±0.005	0.992±0.18	1.53±0.72	ND
11b, White Clover	0.5179±0.07	0.012±0.004	0.468±0.07	0.455±0.05	0.304±0.02

Table2-9. contd

Sample	Pinobanksin	Luteolin	Kaempferol	Pinocembrin	Chrysin+Galangin	Total Conc (µg/g)
1a, Manuka 16+	0.926±0.11	1.05±0.113	0.693±0.09	0.742±0.09	1.08±0.10	8.11±0.81
1b, Manuka 15+	2.40±0.53	0.907±0.16	0.260±0.11	2.75±0.69	2.16±0.51	9.50±2.37
1c, Manuka 12+	0.444±0.01	0.632±0.15	0.402±0.17	0.513±0.15	0.524±0.05	7.32±1.14
2a, Orange blossom	1.68±0.38	0.315±0.10	0.389±0.10	1.33±0.31	0.792±0.24	39.0±10.1
2b, Orange blossom	1.52±0.01	0.077±0.01	1.55±0.11	1.07±0.01	0.465±0.02	26.8±0.21
2c, Orange blossom	0.156±0.07	0.094±0.03	1.09±0.45	0.039±0.01	ND	2.54±1.12
2d, Orange blossom	0.446±0.01	0.07±0.01	3.12±0.16	0.146±0.01	0.079±0.02	12.6±0.62
3, Mangrove	0.508±0.02	0.432±0.07	0.332±0.06	0.198±0.02	0.105±0.03	8.89±1.09
4a, Tupelo	6.07±0.19	ND	2.11±0.05	2.65±0.08	1.73±0.13	60.5±7.4
4b, Tupelo	1.72±0.03	ND	0.610±0.33	ND	0.158±0.01	20.1±1.07
4c, Tupelo	1.74±0.26	ND	4.66±0.42	0.213±0.02	ND	44.0±5.8
4d, Tupelo	4.17±0.39	0.434±0.18	3.02±0.15	2.84±0.25	1.41±0.08	57.5±4.09
4e, Tupelo	2.30±0.16	ND	1.18±0.30	1.63±0.14	0.753±0.05	16.7±2.87
5a, Palmetto	0.832±0.03	0.277±0.06	1.45±0.13	0.064±0.02	0.148±0.04	44.2±1.81
5b, Palmetto	3.71±0.33	ND	0.583±0.14	1.17±0.191	0.701±0.07	40.1±4.75
5c, Palmetto	3.77±1.28	0.386±0.16	1.52±0.52	2.25±0.76	1.25±0.42	21.2±7.33
5d, Palmetto	0.070±0.01	0.415±0.07	0.565±0.10	0.032±0.01	ND	3.19±0.29
6a, Brazilian Pepper	2.06±0.05	0.068±0.00	0.347±0.01	1.48±0.04	1.00±0.07	10.4±0.09
6b, Brazilian Pepper	4.31±0.49	0.733±0.062	0.795±0.09	2.65±0.26	1.96±0.20	14.5±2.22
7, Blueberry	4.56±0.16	0.172±0.023	0.511±0.03	2.63±0.07	1.30±0.09	15.5±0.55
8, Blackberry	0.837±0.64	0.138±0.04	0.678±0.73	0.674±0.07	0.628±0.03	8.86±0.43
9a, Gallberry	0.620±0.07	0.133±0.02	0.391±0.03	0.357±0.04	0.230±0.03	33.8±2.61
9b, Gallberry	2.57±1.07	0.027±0.02	0.322±0.17	0.084±0.02	ND	57.4±24.5
9c, Gallberry	1.88±0.28	ND	0.425±0.04	1.04±0.16	0.476±0.05	42.1±3.9
10a, Avocado	ND	0.345±0.06	0.228±0.05	ND	ND	38.6±5.5
10b, Avocado	ND	0.450±0.23	0.792±0.52	ND	0.035±0.00	10.4±1.30
11a, White Clover	0.798±0.17	ND	0.431±0.10	0.497±0.5	0.370±0.02	4.91±1.11
11b, White Clover	3.00±0.22	0.203±0.01	5.48±0.60	5.44±0.38	2.61±0.10	18.4±1.53

Results are mean ± standard deviation of three determinations on fresh weight basis. ND, not detected. 2-*trans*, 4-*trans*-abscisic acid was quantified using the 2-*cis*, 4-*trans*-abscisic acid as a standard. Pinobanksin was quantified using pinocembrin as a standard.

Table2-10. Content of phenolic compounds in monofloral honeys (µg/g).

Sample	Coumaric acid	Rutin	*2-trans, 4-trans*-abscisic acid	*2-cis, 4-trans*-abscisic acid	Quercetin
12	0.114±0.02	ND	0.488±0.03	1.56±0.09	ND
13	0.139±0.05	0.01±0.00	12.7±2.15	36.4±2.54	0.307±0.09
14	0.083±0.00	0.18±0.01	3.23±0.06	9.39±0.14	ND
15	0.055±0.01	0.084±0.02	1.17±0.17	3.88±0.22	ND
16	0.985±0.08	0.075±0.00	19.2±0.52	32.1±1.23	0.521±0.02
17	2.39±0.33	ND	2.29±0.06	8.95±0.21	0.617±0.02
18	0.987±0.06	ND	7.56±0.12	18.2±0.24	0.215±0.04
19	0.616±0.10	0.044±0.00	0.723±0.23	2.41±0.49	0.578±0.12
20	0.206±0.06	ND	ND	1.52±0.15	0.363±0.01
21	0.188±0.10	ND	0.291±0.05	1.14±0.09	0.192±0.02
22	0.272±0.07	0.030±0.01	9.35±1.1	17.5±1.3	0.557±0.16
23	0.533±0.22	0.054±0.01	5.32±1.57	20.7±2.4	0.494±0.05
24	1.80±0.97	0.101±0.02	2.01±0.08	4.08±0.16	0.410±0.08
25	ND	0.152±0.04	3.78±0.27	10.9±0.77	0.423±0.02
26	1.73±1.06	ND	5.65±0.48	17.5±0.62	0.183±0.08
28	0.171±0.04	ND	9.65±4.02	25.4±5.41	ND

Table2-10. contd

Sample	Pinobanksin	Luteolin	Kaempferol	Pinocembrin	Chrysin+Galangin	Total Conc (µg/g)
12	1.14±0.12	ND	0.199±0.03	0.776±0.13	0.489±0.11	4.92±0.69
13	0.867±0.11	0.263±0.09	0.313±0.15	0.620±0.09	0.439±0.05	96.7±4.8
14	0.199±0.01	0.399±0.03	0.541±0.15	0.053±0.00	0.03±0.00	29.3±11
15	0.691±0.02	0.237±0.01	1.81±0.13	0.395±0.00	0.291±0.04	7.22±0.61
16	4.59±0.07	ND	0.79±0.00	1.88±0.05	1.22±0.03	71.6±2.8
17	3.95±0.12	0.135±0.00	ND	1.51±0.05	1.08±0.04	24.2±1.59
18	4.95±0.07	0.345±0.01	0.290±0.09	3.17±0.08	1.71±0.05	53.6±2.8
19	0.488±0.1	0.196±0.02	0.636±0.12	0.258±0.05	0.159±0.02	3.41±0.99
20	0.385±0.02	0.115±0.00	0.527±0.02	0.219±0.02	0.162±0.00	1.43±0.26
21	0.714±0.03	0.031±0.00	0.322±0.17	0.473±0.02	0.304±0.03	1.93±0.25
22	2.16±0.33	0.226±0.08	1.75±0.50	1.57±0.19	0.775±0.18	37.7±2.4
23	3.57±0.16	0.179±0.00	0.654±0.01	1.69±0.06	1.08±0.06	40.7±4.6
24	1.10±0.05	ND	1.57±0.29	0.208±0.01	0.139±0.02	5.55±0.71
25	ND	0.311±0.07	1.94±0.18	0.124±0.02	ND	12.1±3.8
26	0.471±0.02	0.175±0.01	0.371±0.01	0.154±0.02	ND	23.0±0.69
28	0.442±0.075	0.183±0.03	0.326±0.04	0.489±0.08	0.261±0.01	68.6±2.3

Results are mean ± standard deviation of three determinations on fresh weight basis. ND, not detected. 2-*trans*, 4-*trans*-abscisic acid was quantified using the 2-*cis*, 4-*trans*-abscisic acid as a standard. Pinobanksin was quantified using pinocembrin as a standard.

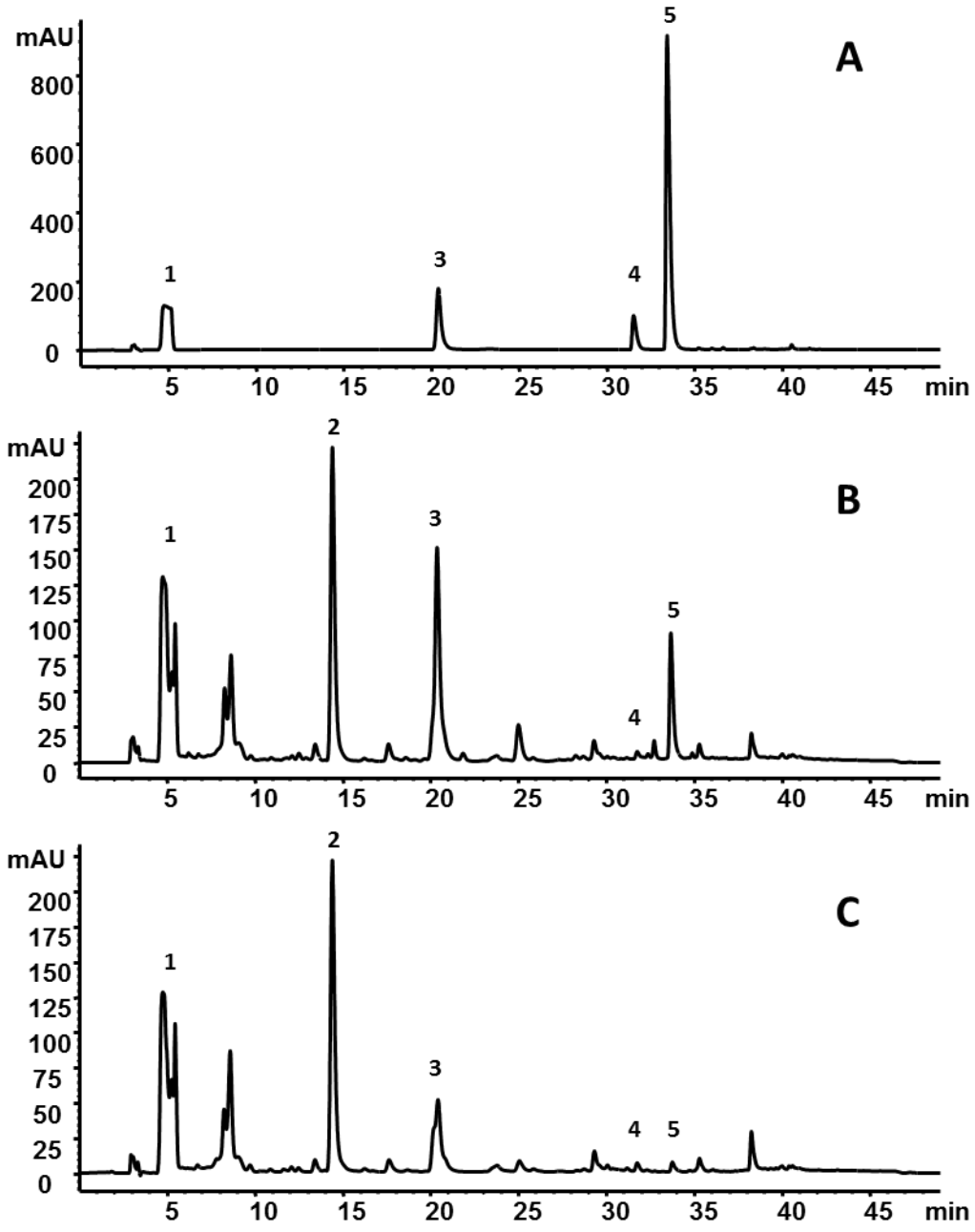

Figure2-1. HPLC chromatogram of α-dicarbonyls in standard solution (A), Manuka honey 1c (B), and White Clover honey 11b (C) after derivatization. 1. *o*-phenylenediamine 2. glucosone 3. 3-deoxyglucosone 4. glyoxal 5. methylglyoxal

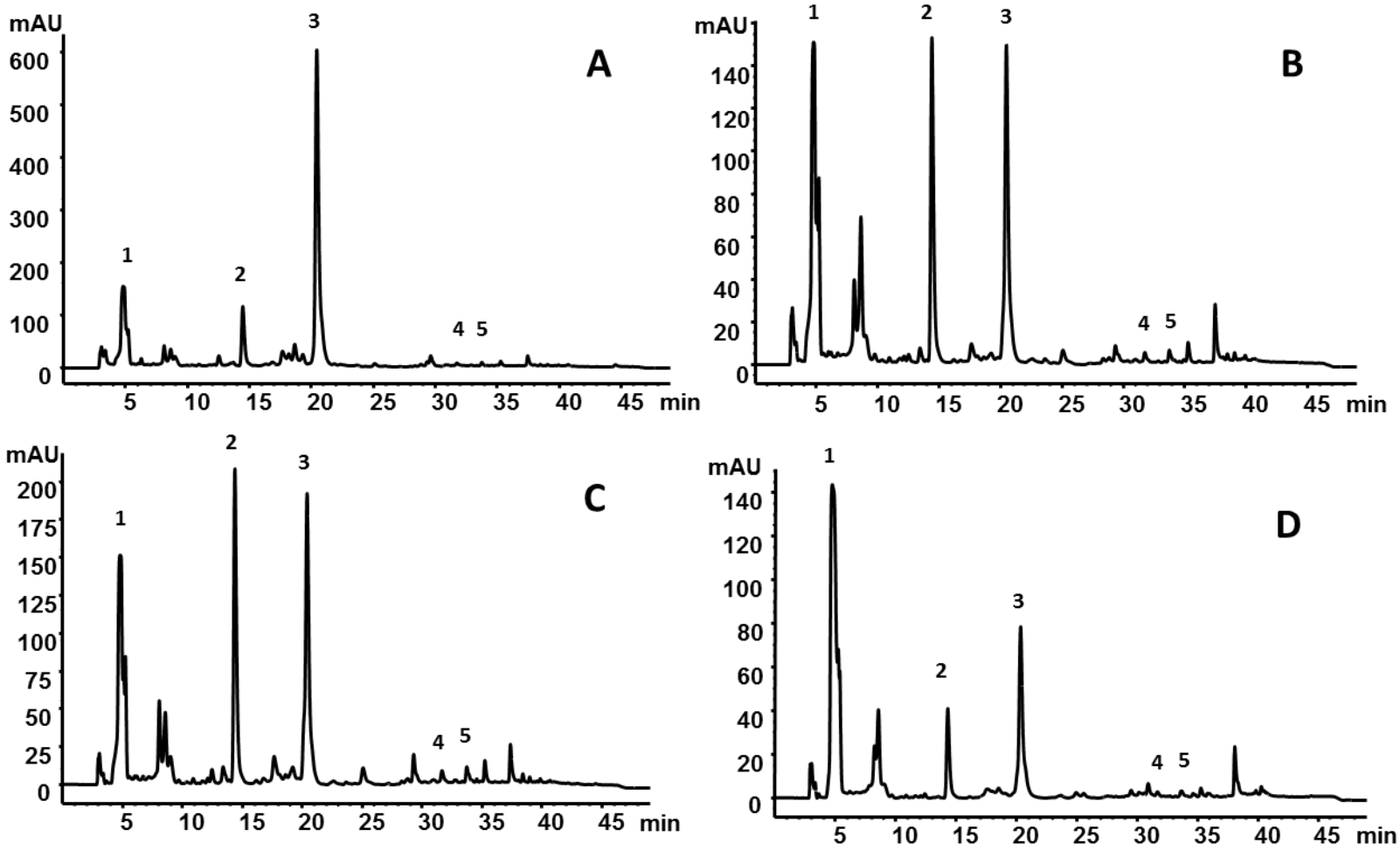

Figure2-2. HPLC chromatogram of α-dicarbonyls in Avocado honey 10a (A), Orange Blossom honey 2a (B), Palmetto honey 5b (C), and Tupelo honey 4b (D) after derivatization. 1. *o*-phenylenediamine 2. glucosone 3. 3-deoxyglucosone 4. glyoxal 5. methylglyoxal

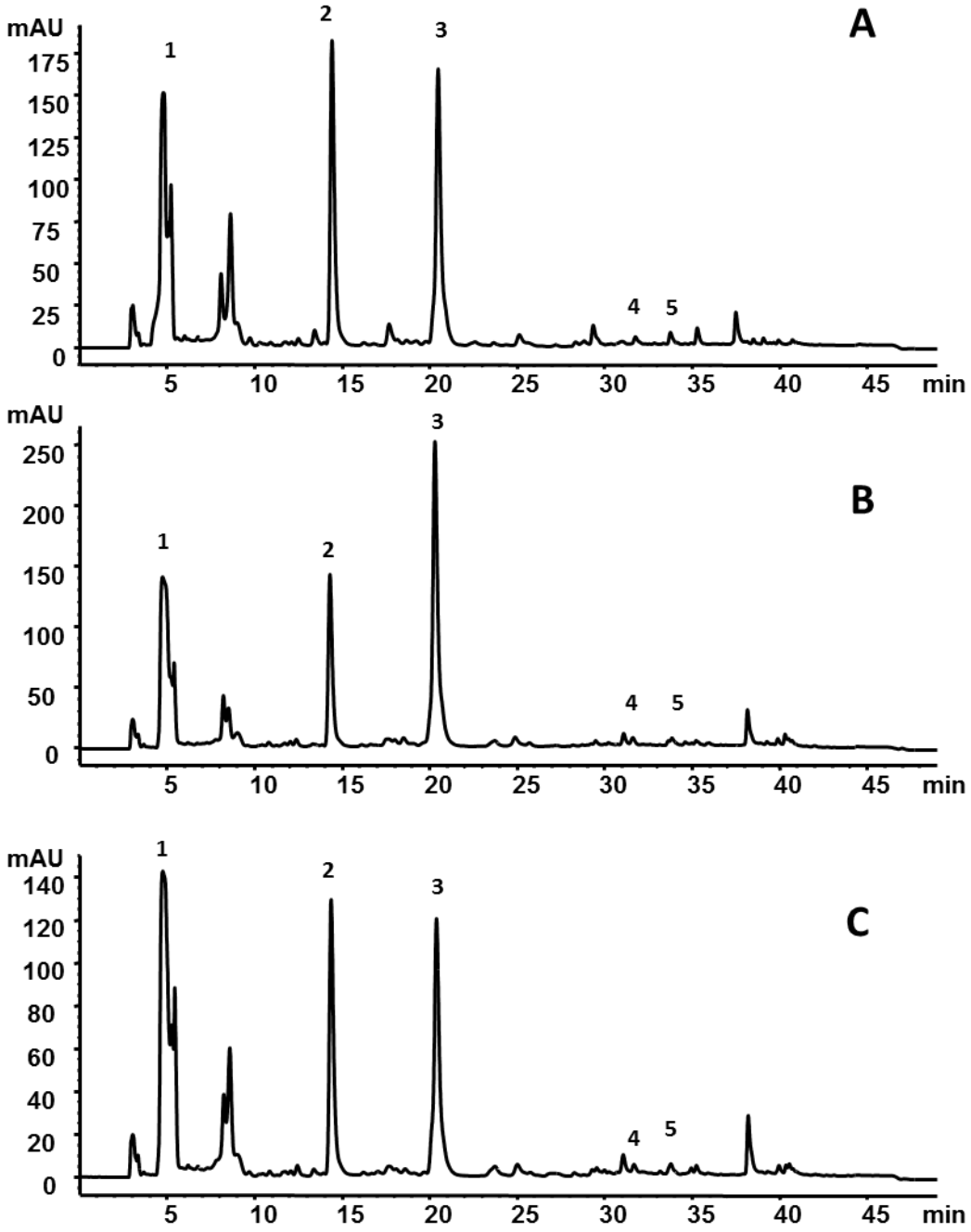

Figure2-3. HPLC chromatogram of α-dicarbonyls in Multi-floral honey 14 (A), Multi-floral honey 23 (B), and Multi-floral honey 25 (C) after derivatization. 1. o-phenylenediamine 2. glucosone 3. 3-deoxyglucosone 4. glyoxal 5. methylglyoxal

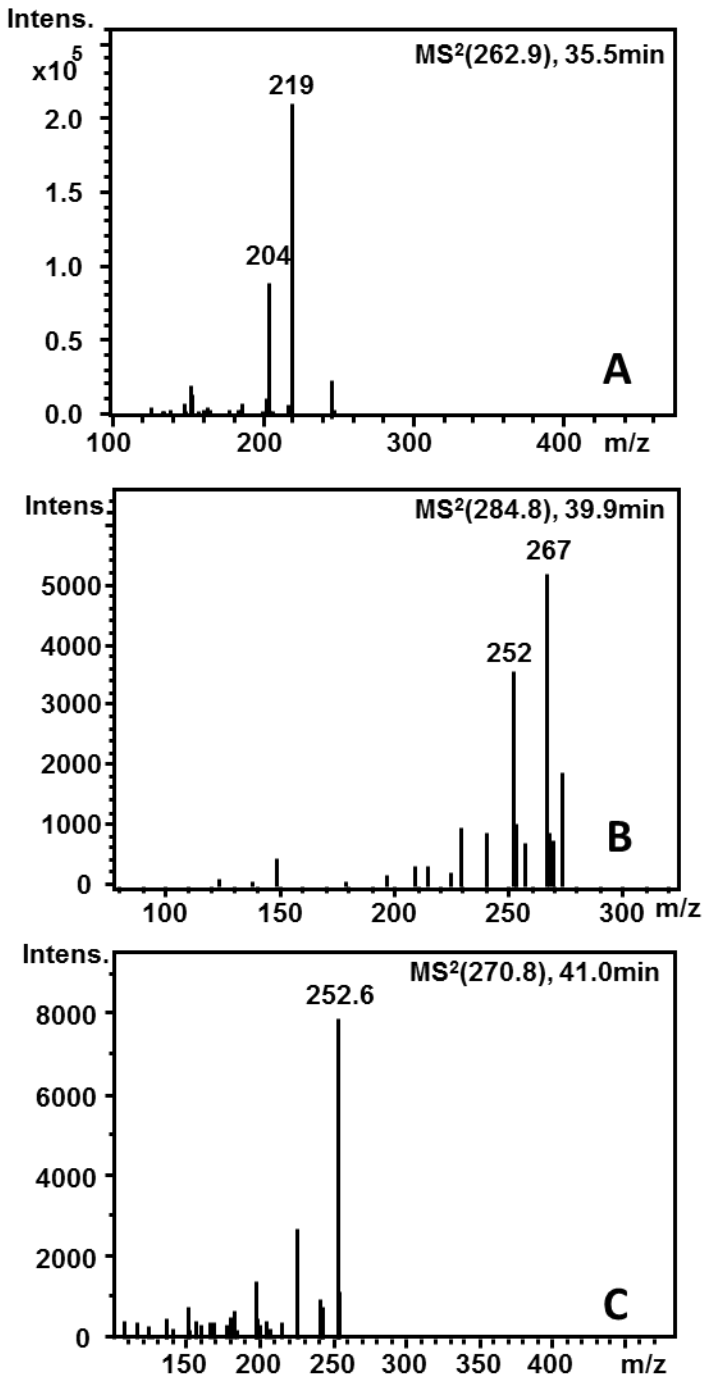

Figure2-4. Product ion spectra (MS^2) of tentatively identified compounds *trans-trans* abscisic acid (A), pinobanksin-5-methylether (B), and pinobanksin(C).

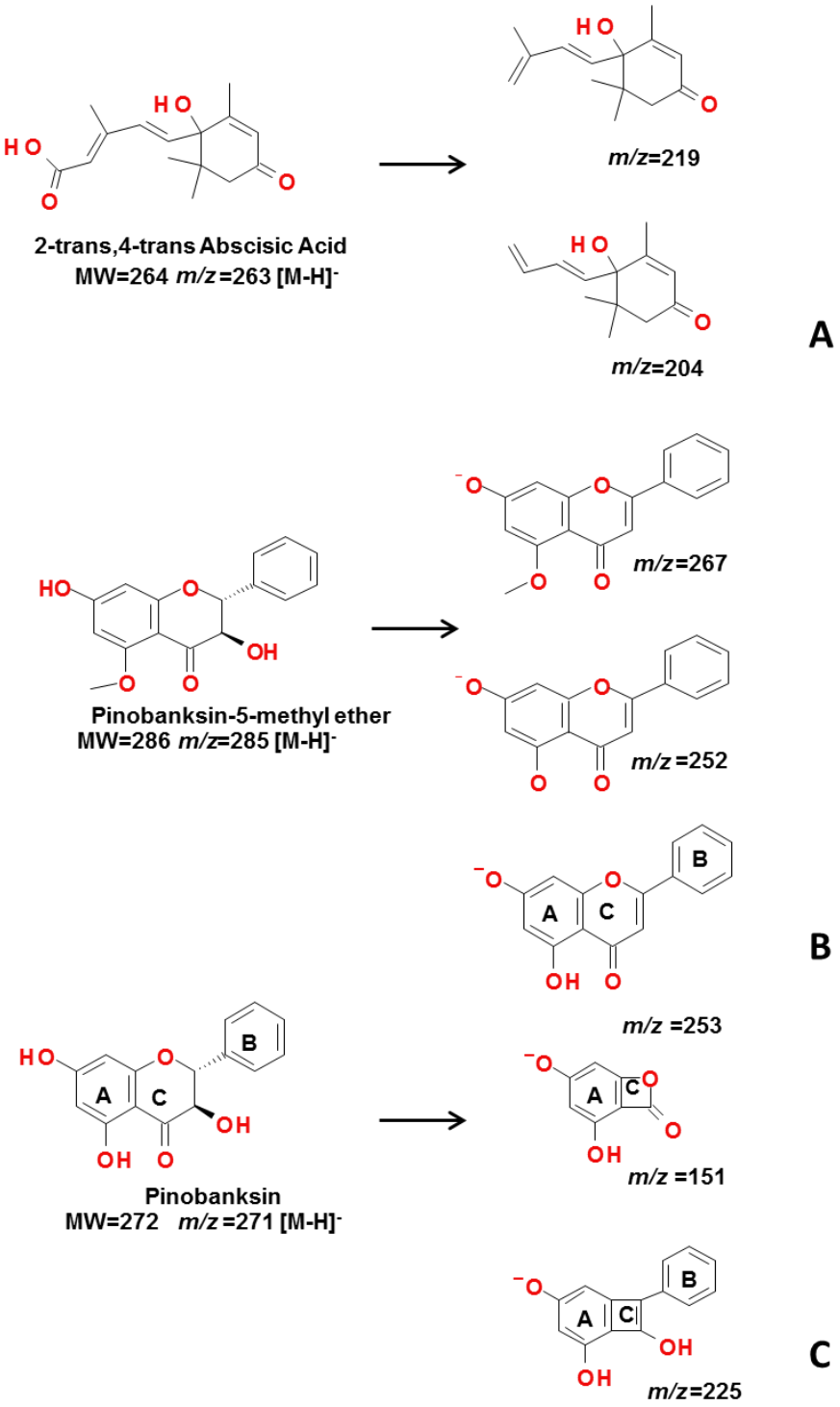

Figure2-5. Proposed fragmentation of tentatively identified compounds 2-*trans*, 4-*trans*-abscisic acid (A) pinobanksin-5-methyl ether (B), and pinobanksin (C).

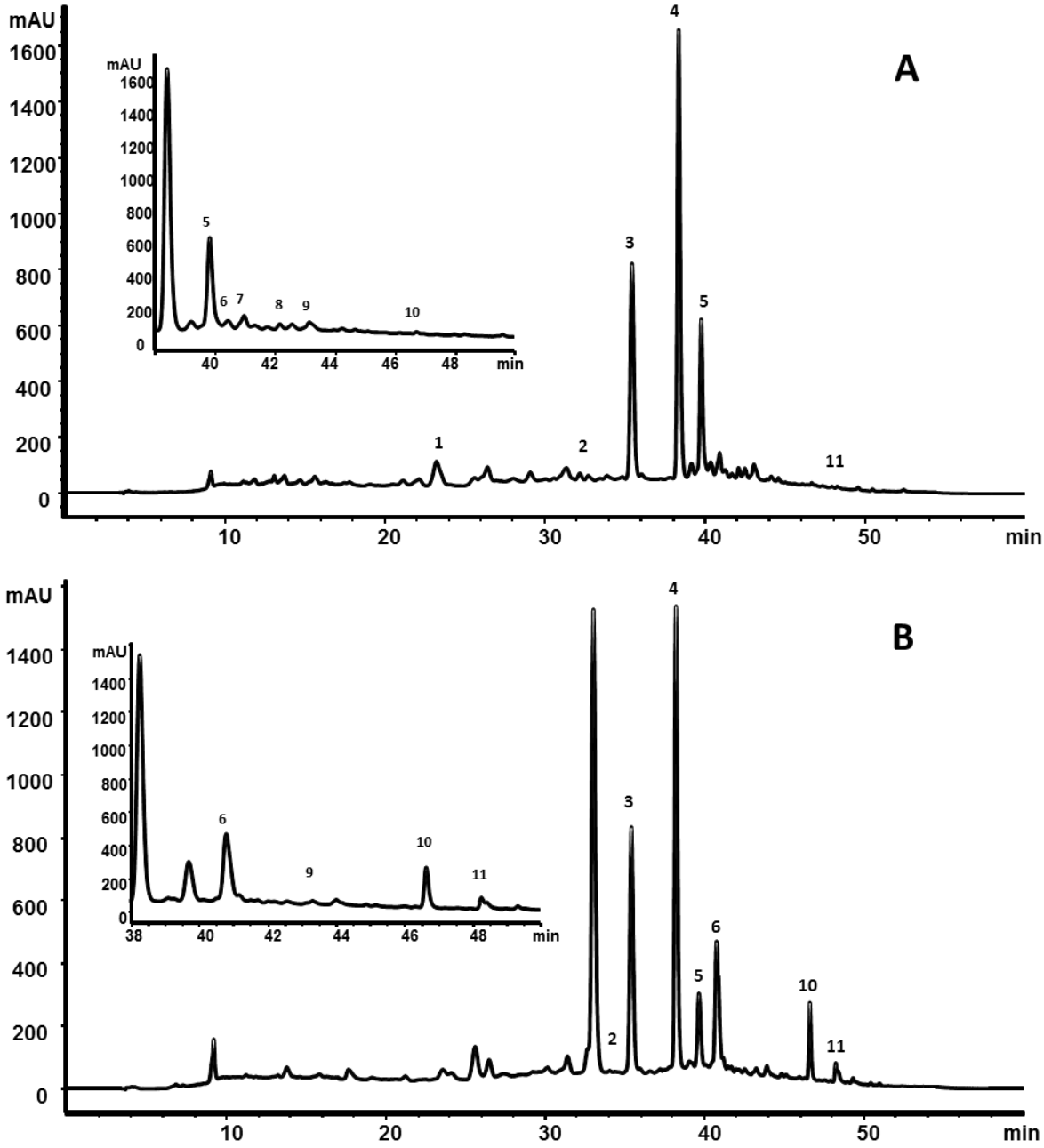

Figure2-6. HPLC chromatogram of phenolic phytocheicals in Palmetto honey 5a (A) and Palmetto honey 5b (B). Peak number and identification are listed in Table 2-8.

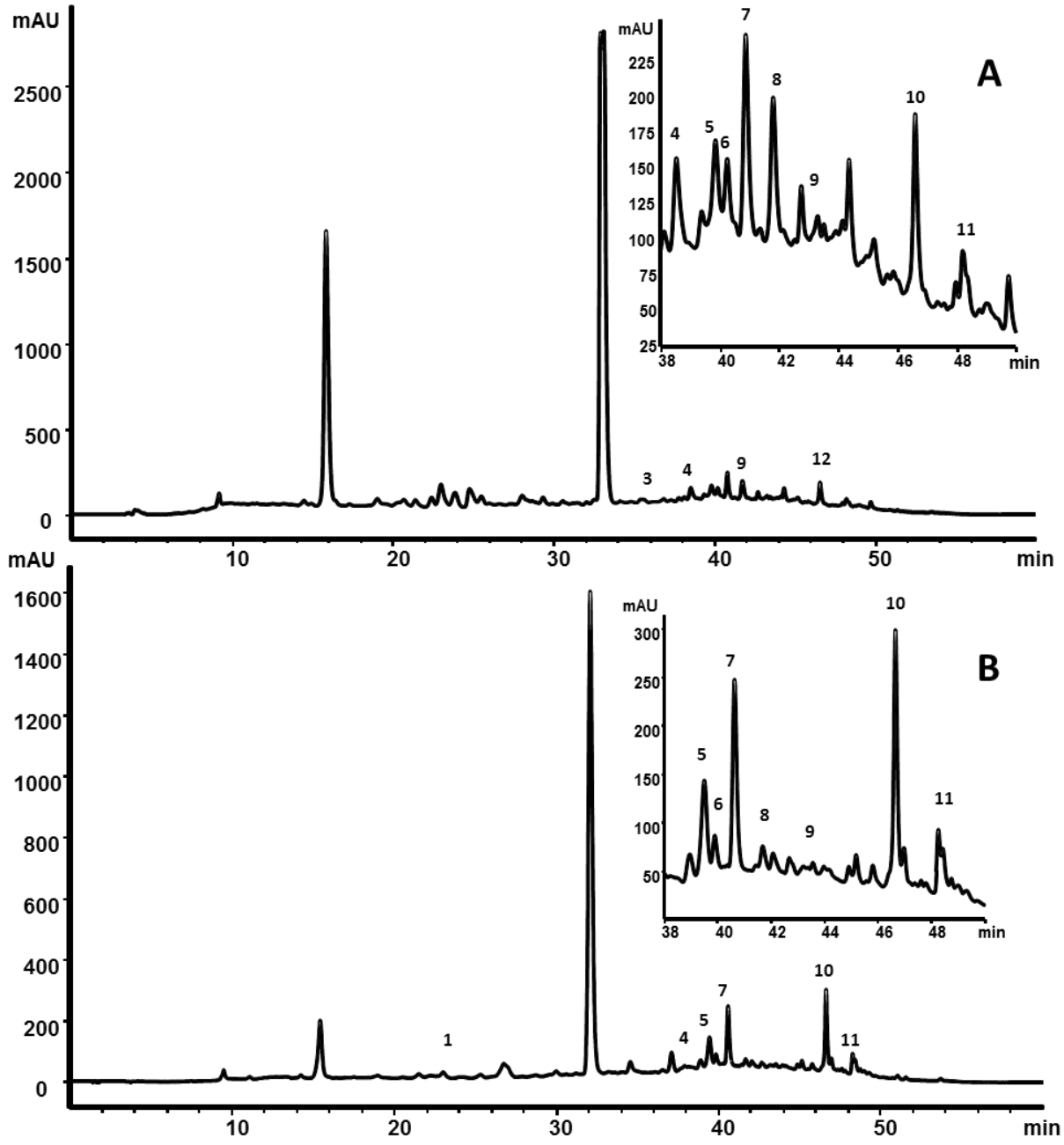

Figure2-7. HPLC chromatogram of phenolic phytochemicals in Manuka honey 1a (A) and Manuka honey 1b (B). Peak number and identification are listed in Table 2-8.

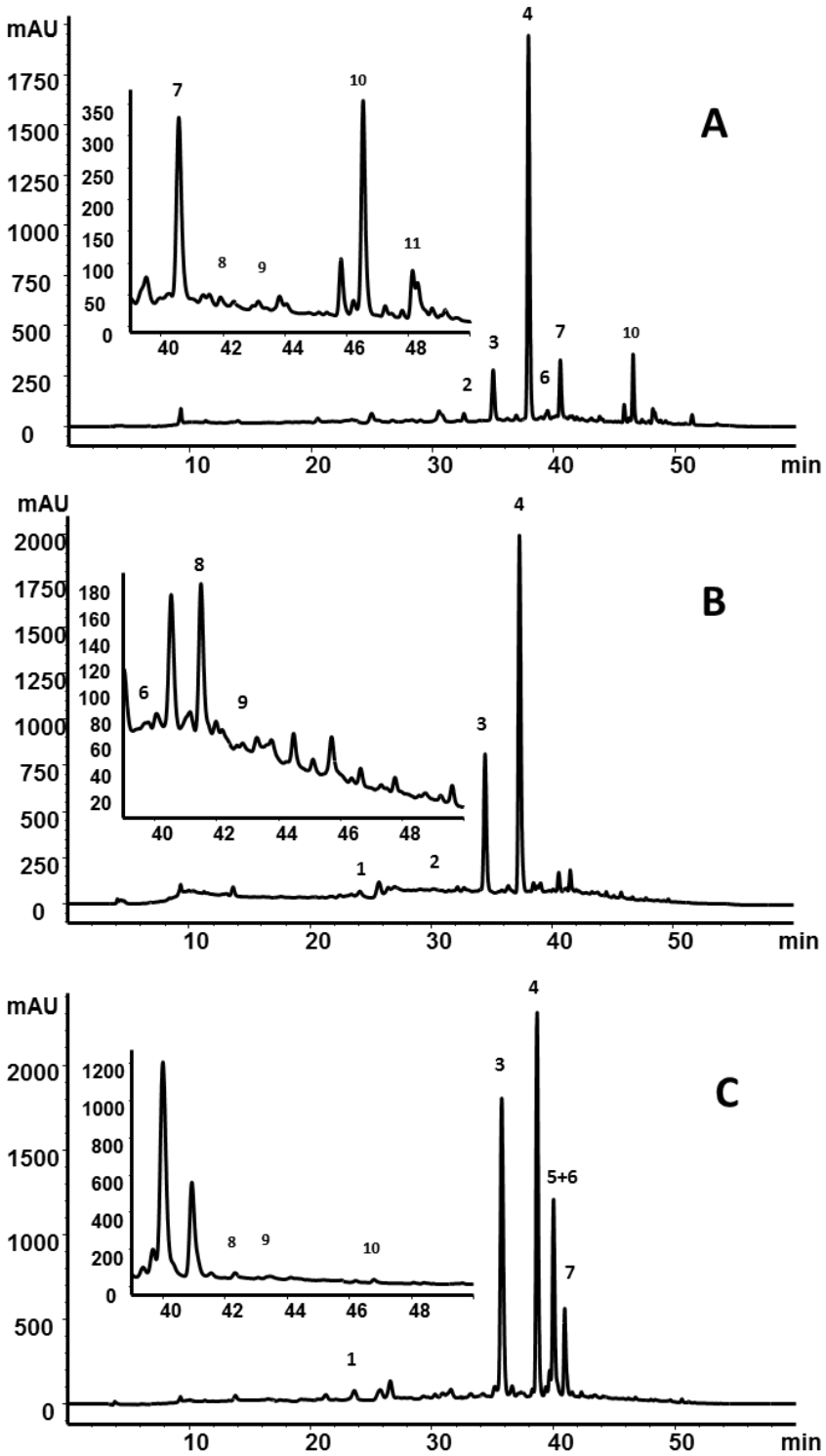

Figure2-8. HPLC chromatogram of phenolic phytochemicals in Orange Blossom honey 2a (A), Avocado honey 10a (B), and Gallberry honey 9b (C). Peak number and identification are listed in Table 2-8.

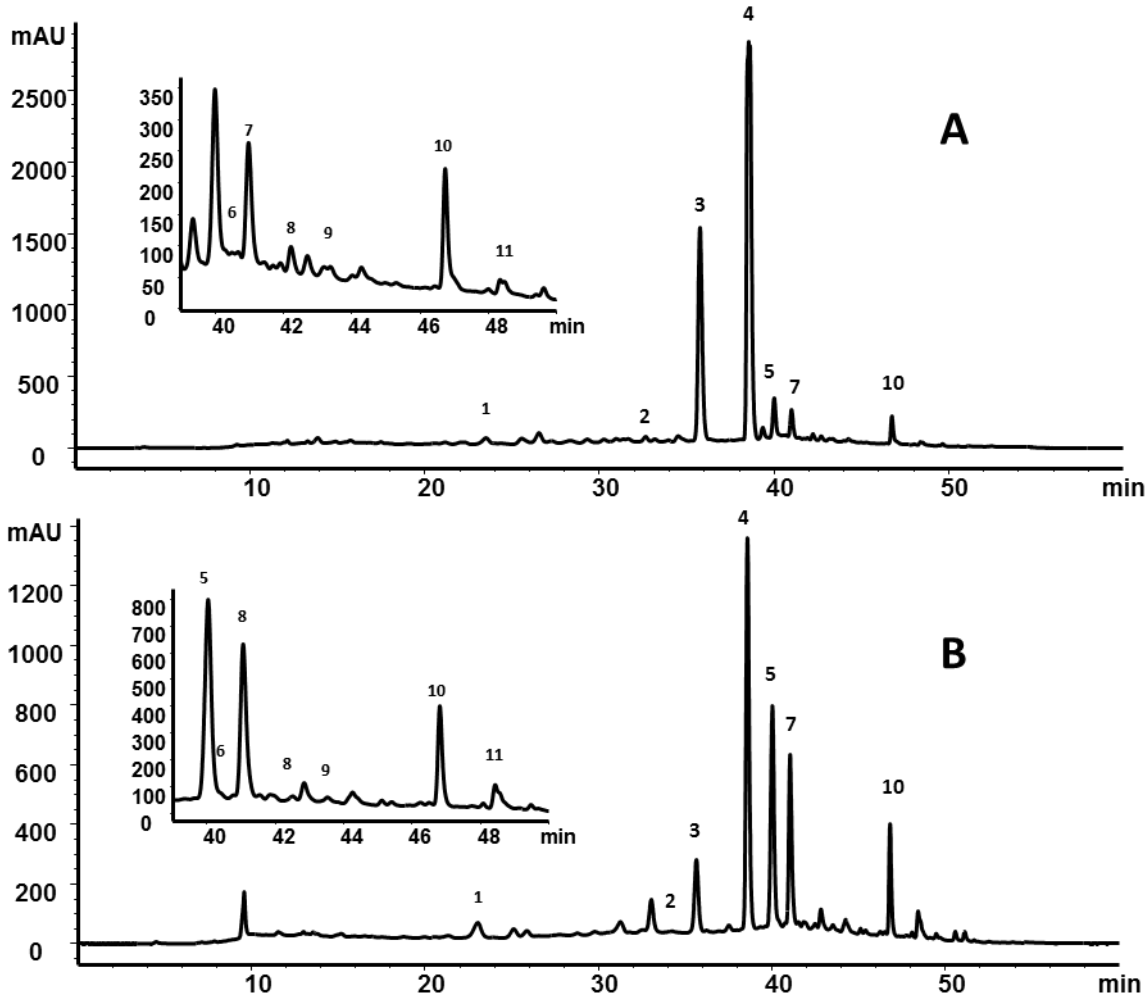

Figure2-9. HPLC chromatogram of phenolic phytochemicals in Multi-floral honey 13 (A), and Multi-floral honey 23 (B). Peak number and identification are listed in Table 2-8.

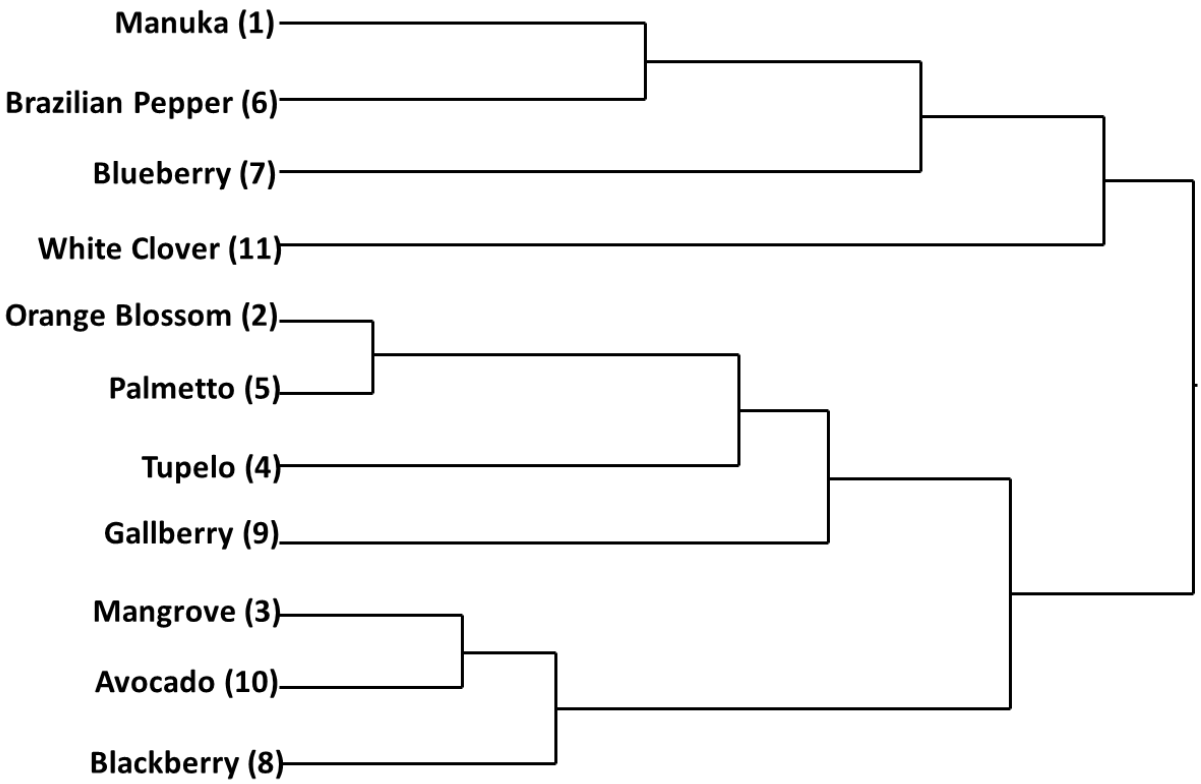

Figure2-10. Hierarchical clustering of monofloral honeys on the basis of phenolic phytochemical composition.

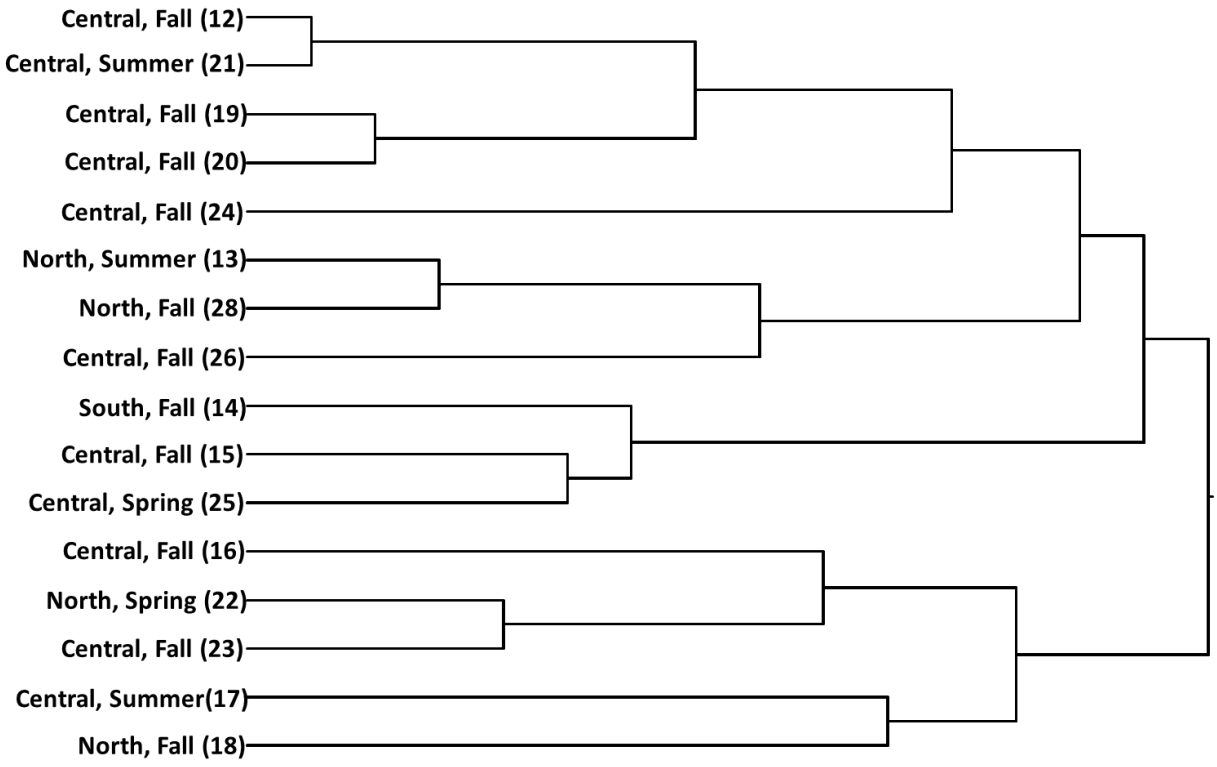

Figure2-11. Hierarchical clustering of multi-floral honeys on the basis of phenolic phytochemical composition. Numbers in parenthesis indicate the sample number.

CHAPTER 3
CONCLUSIONS

Florida honeys contain antioxidant phenolic phytochemicals and anti-microbial α-dicarbonyls. Concentration and composition of these compounds varied according to floral source, harvesting season, and locations. Floral source appears to be the most significant influence on honey characteristics. Results suggest that Florida honey may have medicinal benefits due to their antioxidant capacity and concentrations of beneficial compounds such as flavonoids, phenolic acids and α-dicarbonyls.

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BIOGRAPHICAL SKETCH

Sara Marshall graduated from the University of Florida with a Bachelor of Science degree in food science and human nutrition with a specialization in food science and a specialization in nutrition in May 2011. From there, she accepted a Dean's Graduate Assistantship to pursue her education in food science under the advisement of Dr. Liwei Gu at the University of Florida. Sara graduated with a master's in food science and human nutrition in August 2013. Sara accepted an internship for a governmental research and product development company in Iceland in 2013. Upon completion of her internship she hopes to enter the food industry as a Research Scientist.