PROCESSING TECHNIQUES AND PRODUCTION SYSTEMS ON PHYTOCHEMICAL PROPERTIES IN FRUITS, VEGETABLES AND THEIR PROCESSED PRODUCTS

A Thesis

by

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ABSTRACT

Fruits and vegetables contain substantial amounts of phytochemicals that play crucial roles in the prevention of several chronic diseases. However, there is a general lack of insight among the researchers regarding the impacts of processing techniques, diversity among varieties, and other similar such factors on the overall metabolic profiles of these fruits and vegetables. The recent use of advanced metabolomic approaches in assistance with powerful statistical tools to characterize the entire metabolic composition has been gaining significant momentum in the areas of food science. In the first study, comprehensive metabolomics was combined with chemometric approaches to evaluate the impact of processing techniques on the physiochemical attributes, phytochemical contents and antioxidant activities of 21 commonly consumed vegetables. The results suggested that the purple baby carrot blended juice had the highest total phenolics and DPPH value. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) characterized kaempferol glycosides and betaxanthins attached with amino acids, which, were significantly affected by the processing techniques. In the second study, the chemometric characterization of 30 commercial thermal and cold pressed juices were investigated. Significant variations were observed for metabolic compositions in juices with diverse ingredients. Kaempferol and quercetin glycosides, decarboxylated betalains, and quercetin derivatives were found to be the representative metabolites in classifying the samples. In the third study, the solvent extract efficiency and antioxidant activities in conventionally- and organically-grown beets (red and golden colored) were assessed by using a comprehensive range of solvents. The results demonstrated that red beet extracted with methanol with or without ascorbic acid had the highest betanins, while the water-based extracts had the lowest betanins. Golden colored beet extracted with methanol: ascorbic acid: water (18:80:2, v/v/v) had the highest vulgaxanthin I. Meanwhile, the Ultra-high-

performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) identified 25 phytochemicals in conventional red-, 20 in organic red-, 6 in conventional golden-, and 6 in organic golden-colored beet extracts, respectively. In summary, these results highlight the potential of metabolomic approaches in phytochemical profiling of vegetables grown under diverse production systems and their juices processed through different techniques.

DEDICATION

I would like to dedicate this dissertation and my entire academic experience to my family who taught me the value of education. To my father, Wenbo Wang, who always supported, encouraged, and motivated me. I owe my entire education to his advice and motivation. I know it would be impossible for me to be as selfless person as he is, but I will try my best. To my mother, Haixia Zheng for her love, patience, and encouragement.

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Contributors

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All work conducted for the thesis was completed by the student independently.

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NOMENCLATURE

AA	Ascorbic Acid
ABTS	2,2'Aazinobis (3-etylbenzothiszoline-6-sulphonic acid)
Cyclo-DOPA	Cyclo-dopa 5-O-glucoside
DPPH	2,2-Diphenyl-1-(2,4,6-trinitrophenyl) Hydrazyl
FC	Folin Ciocalteu
HPLC	High Performance Liquid Chromatography
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
L-DOPA	L-3,4-dihydroxyphenylalanine
LC-MS	Liquid Chromatography Mass Spectrometry
TAA	Total Ascorbic Acid
TCEP	Tris (2-carboxythyl) Phosphine Hydrochloride
TP	Total Phenolics
PCA	Principal Component Analysis
PLS-DA	Partial Least Square Discriminant Analysis
VIP	Variable Importance in Projection

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1. INTRODUCTION

Fruits and vegetables consist of several phytochemicals that are a vital part of human diet and nutrition. Given these health-promoting characteristics, some fruits and vegetables are even considered "functional food" by the consumers (Yahia, 2017). Although fruits and vegetables have mounting health benefits, Americans only consume 1.8 cups of fruits and vegetables per day, which is far below the recommended amount (4.5 cups) (Rekhy & McConchie, 2014). Therefore, food manufacturers created beverages with a wealth of functional ingredients to cater consumer needs for "convenient, palatable and health-beneficial" products.

Recently, the general public are more interested in consuming phytochemical compounds derived from food products due to their health-promoting potentials. The use of postharvest processing strategies in retaining and promoting the nutraceutical properties of phytochemicals in processed fruit and vegetable products have become an area of key interest for many researchers (Tiwari & Cummins, 2013). The generally accepted hypothesis was that most of these processing techniques induce rapid enzymatic depletion of natural antioxidants as a result of cellular disruption which allows the contact between substrates and enzymes and lowers the antioxidant concentrations and activities in comparison to the raw samples. The household juicing techniques that are used to extract phytochemicals are generally categorized into blending, centrifugal juicing, and low-speed juicing, based on the type of juicer used. Blenders which are generally used for making smoothies, have blades that chop raw materials to the desired consistency and the pitch of the blades create a kind of tornadic action which circulates the chopped materials into pastes. High-speed centrifugal juicer contains a flat blade disk rotating at high speeds, above which the fruits and vegetables are grounded and filtered. Low-speed juicers employ horizontal helical screws that squeeze juice from the raw materials at low speed and high efficiency (M.-J. Kim, Kim, Kang,

Kwon, Jun, Choi, et al., 2015). Few researches have evaluated the effect of household juicers on quality, free radical scavenging activity, and phytochemical stability, either on a single vegetable or fruit processed products (M.-J. Kim, et al., 2015) (Pyo, Jin, & Hwang, 2014). However, a thorough study on effect of diverse juice processing techniques on phytochemical contents, compositions, and antioxidant activities of several vegetables is required to assay these variables.

Among all the processing techniques, thermal processing is considered the most common technique for processing fruit and vegetable juices due to its convenience and low cost of operation (Odriozola-Serrano, Soliva-Fortuny, Hernández-Jover, & Martín-Belloso, 2009) (Jiménez-Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017). However, thermal processing may have detrimental effects on physical textures, color attributes, sensory qualities and nutritional values of food products. Additionally, increasing consumer demand for safe and naturally processed foods, have intrigued researchers and manufacturers to develop techniques that will have minimal impact on the overall qualities of the processed foods (Bhat & Stamminger, 2015). As a result, alternatives to thermal processing are being sought by food industries. In order to produce food products with desirable sensory characteristics, improved nutritional values, reduced microbial activation, and enhanced health-promoting functionalities, a wide variety of non-thermal technologies, such as high pressure processing (HPP), high pressure homogenization (HPH), irritation, pulsed electric files, ultrasound, supercritical CO_2, ozone and oscillating magnetic field technologies have been used by food industries (Barba, Mariutti, Bragagnolo, Mercadante, Barbosa-Cánovas, & Orlien, 2017a) (Knorr, Ade-Omowaye, & Heinz, 2002) (Pereira & Vicente, 2010). Though the non-thermal processing treatments seem less detrimental than the thermal techniques, the effects are greatly influenced by the food matrix. Therefore, a comprehensive study concerning the effects that these techniques exert on the phytochemical

profiles and antioxidant activities is essential for food manufacturers to design and optimize technological parameters to produce products with balanced quality and sensorial parameters.

Beetroot (*Beta vulgaris* rubra) have been widely used as a natural food pigment and are now ubiquitously added in food products that include yogurts, ice creams, candies, and chewing gums (Chaitanya Lakshmi, 2014). Betalains are major phytochemicals widely found in beetroots and can be divided into red-violet betacyanins and yellow-orange betaxanthins based on the addition of betalamic acid residue attached to the main structure. Numerous studies have shown that betacyanin and betaxanthin have strong antioxidant and anti-inflammatory properties (Khan & Giridhar, 2015) (Strack, Vogt, & Schliemann, 2003) (Clifford, Howatson, West, & Stevenson, 2015). These health beneficial characteristics of beetroot make it a health promoting "functional food" that is widely used in sports drinks and other functional food products (Clifford, Howatson, West, & Stevenson, 2015) (Bazaria & Kumar, 2016). Despite the tinctorial function and health benefits, betalains have limited applications due to their poor stability during processing and storage and warrant a challenge in choosing a suitable solvent system (Celli & Brooks, 2017) (Spórna-Kucab, Ignatova, Garrard, & Wybraniec, 2013). Previous researchers have extracted betalains by solvents with different polarity, type, and ratio, in one variety of beetroot (Fathordoobady, Mirhosseini, Selamat, & Manap, 2016) (Osorio-Esquivel, Álvarez, Dorantes-Álvarez, & Giusti, 2011) (Swamy, Sangamithra, & Chandrasekar, 2014).

In summary, food industries and manufacturers are still facing the challenge of developing or optimizing a processing technique that can provide products with a desired balance between nutritional and sensorial qualities. A bottleneck in the design of such process is the lack of insight into the impact of processing steps on the overall metabolic profile of the fruits and vegetables. In addition, the effect of different processing techniques, production systems, and solvent extraction

systems on phytochemical compositions of fruits and vegetables also remain untapped. Therefore, a comprehensive study investigating the effects of processing techniques, production systems and solvent extract systems on phytochemical profiles and antioxidant activities in different food matrix is much needed. The objectives of this research include:

1) To determine the effect of three juicing techniques on phytochemical contents and antioxidant activities of selected vegetables.

2) To evaluate the influence of thermal and non-thermal processing techniques on the levels and compositions of phytochemicals present in commercial juices

3) To optimize the extraction of betalain compounds and their identification by UPLC-HR-ESI-QTOF-MS in conventionally grown red and golden, and organically grown red and golden colored beetroots.

2. REVIEW OF LITERAUTRE

2.1. Effect of solvent types on betalain compounds

Betalains are hydrophilic compounds whose hydroxyl groups lead to polarity and hydrogen bonding. Extraction solvent has a great influence on betalain extraction procedure and different solvent combinations could affect the extraction efficiency and stability due to intermolecular solute-solvent interactions.

Among all the extraction methods used till now, solid-liquid extraction is the most commonly used method to extract natural pigments (Cardoso-Ugarte, Sosa-Morales, Ballard, Liceaga, & San Martín-González, 2014). Betalain compounds are normally extracted using organic solvents such as methanol, ethanol, acetone, and ethylene or a mixture of these solvents, after which the mixture is normally centrifuged, filtered, and vacuumed (Fathordoobady, Mirhosseini, Selamat, & Manap, 2016). According to Alothman, Bhat and Karim, the mixture of water with ethanol is the most appropriate solvent for polar antioxidants extraction since the extracted product recovers the highest yield of total phenolic content, higher antioxidant activity, and is acceptable for human consumption (Alothman, Bhat, & Karim, 2009). Several authors have previously extracted beetroot and identified betalain compounds by using diverse solvents and methods (Narkprasom, Su, Cheng, Wang, Hsiao, & Tsai, 2012) (T. Kujala, Loponen, & Pihlaja, 2001), whereas the results varied considerably depending on the cultivar, genotype, and phytochemical distribution of the vegetable.

2.2. Effect of processing techniques on phytochemical properties

Previous studies have proposed that processing fruit and vegetables may result in irreversible effects on their sensory attributes, phytochemical profiles and antioxidant activities.

on vegetables. For example, our group (Uckoo, Jayaprakasha, Balasubramaniam, & Patil, 2012) processed grapefruits by three common household processing techniques and demonstrated that hand squeezed fruit juice had significantly higher contents of dihydrobergamottin than the juice processed by juicing or blending. Young-Hee and others (Pyo, Jin, & Hwang, 2014) investigated the influence of processing methods on phytochemical contents and antioxidant activity of blended and juiced Korean kernel fruits (apple, pear, persimmon, and mandarin orange). They concluded that the total polyphenols and antioxidant activities of blended juices were higher than ones processed by juicing. In contrast, ascorbic acid contents in apple, pear, and mandarin orange juices processed by juicing were significantly higher than from blending. Kim and others (M.-J. Kim, et al., 2015) investigated physiochemical properties, phytochemical contents, sensory evaluation and antioxidant activities of tomato juices obtained by high- and low-speed household juicers. They demonstrated that tomato juice obtained from low-speed juicer had better taste, higher phytochemical contents and DPPH values. Mendes Lopes and others (Mendes Lopes, Miguel, Fialho, & Valente-Mesquita, 2016) also investigated color parameters, antioxidant capacity, and microbial stability of grape juice processed by steam extraction, domestic blender, masticating juice extractor, and centrifugal juicer. Juices extracted using steam exhibited higher soluble polyphenols, anthocyanins and higher antioxidant capacity, however, very little information was narrated on phytochemical contents and antioxidant activity of other common vegetables. Thus, it is critical to clarify the effects of thermal and non-thermal processing techniques on physiochemical attributes, phytochemical profiles, and antioxidant activities of commercial and homemade juices.

Food industries are seeking advanced technologies to obtain juices with desirable physiochemical attributes, enhanced nutraceutical composition and potential microbial

inactivation due to increasing consumer demand for minimal and healthy foods. Thermal processing is the most common method for processing food, however, it may induce chemical and physical changes that impair the organoleptic properties and may reduce the content or bioavailability of some bioactive compounds. Therefore, non-thermal processing technologies were established in order to obtain food with "fresh-like" characteristics. Many studies have examined the effect of conventional and non-thermal processing technologies pertaining bioactive content and their products (Rawson, Patras, Tiwari, Noci, Koutchma, & Brunton, 2011). Patras and others (Patras, Brunton, Da Pieve, Butler, & Downey, 2009) processed tomato and carrot purée under thermal treatment (70 ºC/2min) and high pressure treatment (400-600 MPa/15 min/20 ºC), and found that the latter retained more ascorbic acid and possessed stronger antioxidant capacities. Sánchez-Moreno and colleagues assessed the impact of high pressure, pulsed electric files, and traditional thermal processing and observed that high pressure leads to an increase in carotenoid, vitamin A, and naringenin release whereas traditional thermal treatment did not exert any changes in those compounds. In contrast, high pressure did not display higher DPPH radical scavenging activity. Although non-thermal processing juices seem to have more advantages than traditional processed juices, one major concern is that the price of popular nonthermal processed juice (example: cold-pressed juice) tends to be 5 folds higher than traditionally pasteurized juices and this may affect consumer's preference.

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2.3. Variation of phytochemicals in fruits and vegetables

For example, our group (Uckoo, Jayaprakasha, Balasubramaniam, & Patil, 2012) processed grapefruits by three common household processing techniques and demonstrated that hand squeezed fruit juice had significantly higher contents of dihydrobergamottin than the juice processed by juicing or blending. Young-Hee and others (Pyo, Jin, & Hwang, 2014) investigated the influence of processing methods on phytochemical contents and antioxidant activity of blended and juiced Korean kernel fruits (apple, pear, persimmon, and mandarin orange). They concluded that the total polyphenols and antioxidant activities of blended juices were higher than ones processed by juicing. In contrast, ascorbic acid contents in apple, pear, and mandarin orange juices processed by juicing were significantly higher than from blending. Kim and others (M.-J. Kim, et al., 2015) investigated physiochemical properties, phytochemical contents, sensory evaluation and antioxidant activities of tomato juices obtained by high- and low-speed household juicers. They demonstrated that tomato juice obtained from low-speed juicer had better taste, higher phytochemical contents and DPPH values. Mendes Lopes and others (Mendes Lopes, Miguel,

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2.4. Using UPLC-QTOF-HR-MS to identify phytochemicals

In general, mass spectrometry (MS) is a universal technique which is used in the measurement of mass-to-charge ratio (m/z) of ions formed from neutral species in food matrix. Based on their discriminant m/z, these ions are separated after electrostatically directing them into the mass analyzers (Rubert, Zachariasova, & Hajslova, 2015). In 2004, the introduction of ultra-liquid-performance liquid chromatography (UPLC) system with faster separations and increased peak concentrations for qualitative and quantitative analysis of various plant compounds, made the technique extensively preferred to the traditional MS system (Swartz, 2005). Numerous improvements have been opportunely achieved, such as the development and introduction of high-resolution mass spectrometry (HRMS), low-resolution mass spectrometry (LRMS) and modern time-of-flight (TOF) techniques. The combination of UPLC with these advanced techniques allows the complementary performance of specific ion fragmentation as well as the possibility of identification of unknown compounds (Rubert, Zachariasova, & Hajslova, 2015). In addition, it is worth to mention that the coupling of UPLC with HRMS also achieves higher measurement accuracy (within 5 ppm), enhanced sensitivity, improved software handing capabilities, prolonged column lifetime, extended dynamic range and easier mass calibration, which makes it more attractive to the users (Rubert, Zachariasova, & Hajslova, 2015) (Kaufmann, 2014).

Fruits and vegetables and their processed products contain numerous phytochemical compounds that play an important role in reducing the risk or impact of chronic diseases. Recently, the characterization ad differentiation of variety or origin of fruits, vegetables, and derived products by using the UPLC-QTOF-HR-MS have been widely reported. For example, Singh et al. (Singh, Jayaprakasha, & Patil, 2018) performed the rapid UPLC-QTOF-HR-MS technique to identify and quantify the known or novel glucuronide derivatives found in spinach. They found that the 5,3'4'-rtihydroxy-3-methoxy-6:7-methylendioxyflavone-4'-β-D-(2'-Oferuloyl-glucuronide) was the main glucuronide derivative in spinach. Lin and others analyzed the phenolic profiles of red mustard greens (*Brassica juncea Coss Variety*) and efficiently identified 67 anthocyanins, 102 flavanol glycosides, and 40 hydroxycinnamic acid derivatives and provided a database as reference for future analyses (Lin, Sun, Chen, & Harnly, 2011).

2.5. Metabolomics combined with chemometrics in food analysis

Metabolomics is a comprehensive assessment employing advanced approaches and the state-of-art analytical platforms to identify the compositions of small metabolites (< 1500 Da) (A. Zhang, Sun, Wang, Han, & Wang, 2012). Fruits, vegetables and their processed products typically comprise a great varieties of components belonging to diver chemical classes and their wide concentration range (typically from millimolar to femtomolar), makes it a substantial challenge to conduct a comprehensive analysis of the entire metabolic composition (Castro-Puyana & Herrero, 2013). The development of metabolomic approaches has made it possible to profile metabolic constituents in complicated food matrix. Metabolomics consists of targeted (metabolic profiling) and non-targeted (metabolic fingerprinting) analysis. The targeted metabolic analysis focuses on a specific group of metabolites, whereas the non-targeted metabolic analysis is conducted to

develop the patterns of key metabolites that may be responsible for their discrimination (Medina, Pereira, Silva, Perestrelo, & Camara, 2018).

Recently, the UPLC-QTOF-HR-MS has been extensively used in food metabolomics which achieves fast metabolomic analysis (Rubert, Zachariasova, & Hajslova, 2015). In addition, the non-targeted approach is employed with the chemometric tools to assess the key compounds/metabolites. Chemometrics is a statistical tool that extracts meaningful information from a large amount of data in the targeted and non-targeted approaches, to identify food components (Esteki, Simal-Gandara, Shahsavari, Zandbaaf, Dashtaki, & Vander Heyden, 2018). Marsol-Vall et al. (Marsol-Vall, Balcells, Eras, & Canela-Garayoa, 2018) optimized an effective Stir Bar Sorptive Extraction (SBSE) method combined to the GC-MS and chemometric to classify four varieties of peach juices under different processing conditions. The methodology included metabolomic profiling of the juices followed by chemometric tools to analyze the profiles of these metabolites. The PCA and SLDA successfully separated and characterized 14 variables under different chemical categories such as lactones, fatty acids, fatty aldehydes, hydrocarbons, and alcohols, which are mainly responsible for the sample separation, thus implementing the future use of SBSE technique in discriminating liquid samples.

The systems behind the production of different crops could potentially influence the levels and composition of certain phytochemicals, which may result in the identification of food production biomarkers. In this context, several studies have assayed the influence of metabolic profile of different food stuffs (food metabolome) (Krejčová, Návesník, Jičínská, & Černohorský, 2016), (dos Santos, Lima, dos Santos, Silva, de Santana, de Araujo, et al., 2019), (Hohmann, Monakhova, Erich, Christoph, Wachter, & Holzgrabe, 2015). This discussion is particularly attributed to increasing consumer's interest in organic foods.

The food metabolome can also be affected by the home-scale or industrial processing techniques. A recent study evaluated the impact of different industrial processing manners (blending and heating order) on the phytochemical levels and compositions in carrot, tomato, and broccoli purees (Lopez-Sanchez, De Vos, Jonker, Mumm, Hall, Bialek, et al., 2015). By performing a hybrid of targeted and untargeted metabolomic approaches such as 1H NMR and LC-QTOF MS, different key metabolites were identified that significantly affected the product quality. In addition, the results proved that the antioxidant compounds such as vitamin C and vitamin E, flavor and fragrance components like hexanal and decadienal are also influenced by the heating-blending order. Similar study has also been conducted to compare the effect of industrial and home processing techniques by employing untargeted LC-QTOF-MS analysis with PCA analysis (Tomas, Beekwilder, Hall, Sagdic, Boyacioglu, & Capanoglu, 2017). The PCA graph clearly separated the industrially and home processed samples, with the industrially processed samples having higher antioxidant activities. Additionally, during food processing the chemically modified metabolites were possibly obtained. Therefore, the food processing enriches the chemical pattern and could be perceived as reference for food authentication (Rubert, Zachariasova, & Hajslova, 2015).

3. COMPREHENSIVE METABOLOMICS COMBINED CHEMOMETRICS APPROACH TO EVALUATE THE IMPACT OF THREE PROCESSING TECHNIQUES ON THE PHYTOCHEMICAL PROFILES AND ANTIOXIDANT ACTIVITIES IN COMMONLY CONSUMED VEGETABLES

Epidemiological studies have shown that excessive generation of reactive oxygen species (ROS) causes systemic oxidative stress, induces damages to cellular lipids, proteins, or DNA functions, and ultimately results in human diseases such as cardiovascular diseases, kidney disease, diabetes, cancers, strokes, inflammations, infections, retinal damages, and arthritis.(Forbes, Coughlan, & Cooper, 2008; Pham-Huy, He, & Pham-Huy, 2008) Recent literatures have substantially investigated the correlations between the regular consumption of vegetables and the reduced risk of various oxidative stress.(Williams, Edwards, Hamernig, Jian, James, Johnson, et al., 2013) The beneficial potentials of vegetables are attributed to the presence of natural antioxidants in vegetables, such as vitamins, minerals, phenolics compounds, alkaloids, and other nitrogen-containing plant constituents that act as free radical scavengers in human bodies,(Dillard & German, 2000; Nile & Park, 2014) while the composition and concentrations of phytochemicals are greatly varied in the specie, genotype, and variety of the vegetables.(Dillard & German, 2000; Nile & Park, 2014)

Kale, cauliflower, and turnip are the commonly consumed vegetables of Brassicaceae family that are rich in natural antioxidants especially phenolic compounds.(Cartea, Francisco, Soengas, & Velasco, 2011) Phenolic compounds are plants secondary metabolites synthesized by phenylalanine through shikimic acid pathway and categorized as free, esterified and insoluble-bound forms.(Shahidi & Yeo, 2016) In *Brassica* vegetables, flavonoids (flavanols and anthocyanins), hydroxycinnamic acid and phenolics are in the most abundant

concentrations.(Cartea, Francisco, Soengas, & Velasco, 2011) Carrots (*Daucus carota* L.) are economically important crop that are generally revered as "good for eyes" due to the presence of carotenoids. Recently, packaged baby carrots of various colors such as purple, yellow, white, and orange (rainbow carrots) are gaining popularity due to the convenience for consumption and attractive colors.(Arscott & Tanumihardjo, 2010) Rainbow carrots also have a wide variety of phytochemicals including carotenoids, phenolics compounds, and dietary fibers, which make them ideal functional food. Beetroot (*Beta vulgaris* L.) is a commonly consumed vegetable and ranked among the 10 most powerful vegetables int terms of their antioxidant activities, which is attributed to the presence of betalains, phenolics compounds, and other phytochemicals.(Vulić, Čanadanović-Brunet, Ćetković, Tumbas, Djilas, Četojević-Simin, et al., 2012)

Despite their health promoting potentials, the unpalatable flavors and lesser convenience in consumption, often tends people to process vegetables into juices. However, this process may affect the phytochemicals present in them and their antioxidant activities by exposing the inner tissues to oxygen and light.(Tiwari & Cummins, 2013) Meanwhile, food processors are trying to optimize processing steps in order to prevent phytochemicals from reducing or losing their nutraceutical and pharmacological properties. Several studies have been devoted to investigating the effect of processing techniques on the overall antioxidant activities and phytochemical content in one particular fruit or vegetable, and the results are inconsistent or contradictory.(M.-J. Kim, et al., 2015; Mendes Lopes, Miguel, Fialho, & Valente-Mesquita, 2016; Pyo, Jin, & Hwang, 2014) Therefore, the composition and abundance of characteristic phytochemical compounds of low molecular weight (< 1500 Da) is affected by diverse processing techniques, and as only few reports are available in the literature, a reliable and consistent technique is needed.

Metabolomics is a functional approach that systematically identifies and quantifies numerous targeted and untargeted small metabolites present in food samples.(Castro-Puyana & Herrero, 2013) The rapid growth of metabolomics has been attributed to the substantial development of modern analytical techniques. In particular, the introduction of ultrahigh pressure liquid chromatography (UHPLC) employs porous particles with a small internal diameter (< 2μm), combined with ion-trap-time-of-flight (QTOF) platform, allowing a broader analysis of compounds, obtaining higher peak capacity, enhanced resolution and enhanced sensitivity compared to the conventional HPLC columns, and results in faster separation of multiple compounds.(Castro-Puyana & Herrero, 2013) The data obtained from such analytical techniques by the metabolomic approach needed to be properly processed and interpreted by the use of cutting-edge data processing software and multivariate chemometric tools. Principle component analysis (PCA) is one of the most frequently used unsupervised method for sample clustering. Partial least squares discriminant analysis (PLS-DA) is widely applied as a supervised technique for construction of classification and prediction models.

In this study, we aimed to compare the effects of three household-scale juicing techniques; blending, high-speed centrifugal juicing, and low-speed juicing on the composition and levels of phytochemicals of 21 commonly consumed vegetables. Using an untargeted UPLC-HR-ESI-QTPF-MS analysis, a number of phytochemicals presented in processed vegetable juices were monitored and identified by comparing their retention time, exact mass and, characteristic fragmentation with the information available in databases. Then, the targeted metabolomics was combined with chemometric tools to identify the characteristic components responsible for differentiating the phytochemical profiles under the three processing techniques. In addition, the

color attributes, phytochemical contents, and antioxidant activities of the processed juices were also determined.

3.1. Materials and methods

3.1.1. Chemicals

L-ascorbic acid, gallic acid, phosphoric acid, sodium carbonate, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), Folin Ciocalteu (FC) reagent, 2,2´-azinobis(3-etylbenzothiszoline-6-sulphonic acid) diammonium salt (ABTS), α-amylase, D-glucose, starch from potato, methanol and HPLC grade acetonitrile, were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents were analytical grade. Nanopure water (NANOpure, Barnstead, Dubuque, IA) was used for the entire study.

3.1.2. Sample preparation

Twenty-one vegetables with different varieties, productions systems, or colors were purchased from local markets. Vegetables include white, yellow and green cauliflowers (*Brassica oleracea var. botrytis*); green, green organic, red organic, and black kales (*Brassica oleracea var. sabellica*); purple-white and white baby turnips (*Brassica rapa subsp. rapa*); red, green (Watermelon) and white (Daikon) radishes; red, red organic, golden and golden organic beetroots (*Beta vulgaris*); orange, purple, yellow and white of baby carrots (*Daucus carota subsp. sativus)*. Detailed information is available in Table A-1. All vegetables were thoroughly washed, dipped into nano pure water, and then wiped completely dry. Each individual vegetable was cut into 1.5 cm × 1.5 cm piece and equally divided into three groups. Each group of samples was processed by an Osterizer 12-speed Blender (blending), Breville Juice Fountain Plus 850-watt 2-speed juice

extractor (high-speed centrifugal juicing), and Omega 8006 Nutrition System HD Low Speed Juicer (low-speed juicing), respectively.

3.1.3. Instrumental color attributes

Color attributes of each sample were measured by a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan). The instrument was calibrated using a white plate (Calibration Plate CR-A43, Minolta Cameras, Osaka, Japan) to standardize the equipment. Afterwards, the processed vegetable juices were transferred into a glass cuvette for measuring the Hunter Lab units L, a* and b* with the L indicates lightness (0 = black, 100 = white); a* represents redness-greenness (positive = red, negative = green); b* indicates yellowness-blueness (positive = yellow, negative = blue). The samples were filled in taking care of all air bubbles. Triplicate measurements were conducted, and the results were expressed as mean ± standard error.

3.1.4. Targeted phytochemicals quantification

Total ascorbic acid content was determined using our developed method (Chebrolu, Jayaprakasha, Yoo, Jifon, & Patil, 2012a). Briefly, 4.0 mL of processed vegetable juice was added to 4.0 mL of 3% meta-phosphoric acid (3 g dissolved in 100 mL of nanopure water), and the mixture was vortexed (30 s), homogenized (9000 rpm for 2 min), sonicated (1h), and centrifuged (9000 for 15 min) to obtain the supernatant. Further, 1mL of the supernatant was centrifuged at 10000 rpm for 5 min and 400 µL of the clean liquid mixed with 400 µL Tris (2-carboxyethyl) phosphine hydrochloride reagent (TCEP) was pipetted into HPLC vials. For HPLC analysis, an Agilent 1200 Series HPLC System, equipped with an analytical C18 column (250 mm × 4.6 mm, 5 µm) and a photodiode array detector (PDA), was performed with an isocratic elution of

phosphoric acid as a mobile phase. A 10 μL of sample was injected to the column with a flow rate of 1 mL/min, and the peaks were monitored at 254 nm. Results were expressed as μg of ascorbic acid per g of fresh weight.

Analysis of nitrate was conducted by mixing 1mL of sample juice added 8 mL of nanopure water. The mixture was vortexed (30 s), homogenized (9000 rpm for 2 min), sonicated (1h), and centrifuged (9000 for 15 min) to acquire the supernatant. After that each sample was further filtered with 3 mL syringe (BD, Franklin Lakes, NJ, USA) and filter (VWR International, Radnor, PA, USA). Agilent 1200 Series HPLC System was used to identify and quantify nitrate in samples. Injection volume was 10 μL with flow rate set at 0.7 mL/min and wavelength at 210 nm. Mobile phase was 30 mM phosphoric acid solution with an isocratic solution. Results were expressed as μg of nitrate per g of fresh weight.

3.1.5. Estimation of total phenolics

3.1.5.1. Sample extraction

Five milliliters of the obtained juice were first mixed with 10 mL of methanol, then the mixture was placed in the vortex for 30 s. After homogenizing the mixture at 9000 rpm for 1 min, the mixture was sonicated under ice for 1h. Afterwards, the mixture was centrifuged at 10000 rpm for 10 min and then collect supernatant. The residues were added into 3 mL methanol and centrifuge at 10000 rpm for 10 min to collect supernatant. Final volume was calculated by adding the two-supernatant collected as mentioned above.

3.1.5.2. Estimation of total phenolics

A Folin-Ciocalteu colorimetric method was adopted to spectrophotometrically measure the total phenolics (G. Jayaprakasha & Patil, 2007). The absorbance was set at 734 nm using a KC-4 Microplate Reader (Bio Tek Instruments, Winooski, VT, USA) Data were expressed as μg gallic acid equivalents (GAE)/g FW.

3.1.5.3. Untargeted UPLC-HR-ESI-QTPF-MS based identification

The methanol extract of each sample was analyzed by LC-MS. UPLC-HR-ESI-QTPF-MS analysis for phenolic compounds in the processed vegetables samples was performed on a 1290 Agilent HPLC LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a maXis impact mass spectrometer (Bruker Daltonics, Billerica, MA, USA). An eclipse plus C18 column (1.8 μm, 50 × 2.1 mm; Agilent) was used for phytochemical compounds separation with the binary mobile phase consisting of 0.2% formic acid (A) and 0.1 % formic acid in water: acetonitrile (3:7, v/v) with gradient elution as follows, 100% A for 0 5 min, 100-98% A for 5 min, 100-98% for 5 to 10 min, 98-55% A from 10 to 22 min, 55-44% A from 22 to 27 min, 40-10% A from 27 to 31 min, isocratic for 31 to 34 min, 10-100% A from 34 to 40 min. Capillary voltage was set at 40 kV and nebulization pressure was 241.3 kPa. Nitrogen temperature was 350 °C and flow rate was 8.0 L/min. The column temperature was set at 65 °C. Injection volume was 10 μL and the flow rate was 0.2 mL/min. For MS spectral analysis, the following mass spectrum detection conditions were employed in positive mode with MS scan range 50-1000 *m/z*. The accurate mass data for were processed using he Data Analysis 4.3 software, and identification for each metabolite was performs by comparing the mass accuracy, isotopic patterns, adducts, and fragments using SmartFormaula.

3.1.6. Antioxidant activity

3.1.6.1. DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed according to the method outlined by our published protocol (Bae, Jayaprakasha, Jifon, & Patil, 2012) with slight modifications. The DPPH radical scavenging activity was calculated by using a calibration curve of a series of ascorbic acid solutions and expressed as μg ascorbic acid equivalents per g for fresh weight for each vegetable (μg AA/g FW).

3.1.6.2. ABTS free radical scavenging activity

The ABTS (2,20 -Azinobis-3-ethylbenzotiazoline-6-sulphonic acid) assay was conducted according to our published protocol (G. K. Jayaprakasha, Girennavar, & Patil, 2008). The absorbance was measured at 734 nm and results were expressed as μg ascorbic acid equivalents per g for fresh weight for each vegetable (μg AA/g FW).

3.1.6.3. α-Amylase inhibition assay

In the α-Amylase inhibition experiment, 10 μL of the extracted sample was mixed with saline solution (0.9 %) to obtain a volume of 140 μL. After adding 45 μL of starch (10 mg/mL) and 45 μL of α-amylase (10 mg/mL), the microplate was incubated at 25 C for 1 h. Then, 50 μL of DNSA color reagent solution (96 mM 3,5-dinitrosalicylic acid) was added into a 96-well plate. Then, the microplate was placed into a 75 °C oven for further reaction. The absorbance of the reaction mixture was measured at 540 nm. To eliminate the background absorbance produced by

sample extracts or fractions, an appropriate extract control without enzyme was included. The results were expressed as D-glucose equivalents (μg) of fresh weight (g).

α-Amylase inhibition% = [1-（A_{sample}- A_{blank} /A_{test} – $A_{control}$ ）]100%

where A sample is the absorbance of the mixture of the sample, starch solution, enzyme and DNSA color reagent solution; A blank is the absorbance of the mixture of phenolic sample, starch solution and DNSA color reagent without enzyme; A test is the absorbance of the mixture of buffer (instead of sample), starch solution, enzyme and DNSA colour reagent; A control is the absorbance of the mixture of buffer, starch solution and DNSA color reagent without enzyme.

3.1.7. Data processing and chemometrics analysis

All samples were analyzed in triplicate and the results were express as the mean ± standard error. For comparisons with samples, data was analyzed by one-way ANOVA and Tukey's multiple comparison test using JMP software (SAS, NC, USA). A probability of 5% or less was accepted as statistically significant. All multivariate data analysis was carried out using MetaboAnalyst software (http://www.metaboanalyst.ca/) to detect clustering and discriminate samples in the present study.

3.2. Results and discussions

3.2.1. Effect of household processing techniques on color attributes

When choosing a food product, color is the first attribute perceived by consumers which directly affects the acceptability and their purchasing preference. In a food matrix, color is also considered as an important indicator of food quality and occurrence of chemical or biochemical

reactions (Patras, Brunton, O'Donnell, & Tiwari, 2010). Previous literature has proved that the colors present in vegetable juices are mainly due to the presence of phytochemical pigments, such as chlorophylls, carotenoids, anthocyanins and betalains (Barrett, Beaulieu, & Shewfelt, 2010). In our study, the L (brightness), a*(redness-greeness), b*(yellowness-blueness) color space coordinated of the vegetable juices obtained by three processing techniques (blending, high-speed centrifugal juicing, and low-speed juicing) are shown in Table A-1. The vegetable juices obtained through blending had lower L values than juices in comparison to the juices obtained through high-speed centrifugal juicing and low-speed juicing, indicating that dark color was formed during blending. The blender crusting the whole vegetable using the high-speed spinning blades, generating heat and initiating serial biochemical reactions and color changes. In addition, the sediment and paste retained after processing could also cause dark color in the blended juice. In contrast, juices obtained by high and low-speed juicing were translucent, bright, and no heat was generated during the process, which, made the juices similar to industrialized juice products. However, red organic kale, golden beet, and purple baby carrot were the exceptions, where the blended juices were brighter than their counterparts.

3.2.2. Variation in phytochemical concentration under three processing techniques

In our study, total ascorbic acid (TAA) and nitrate content of the 21 vegetables juices are given in Table 2. Among all the vegetables, white cauliflower had the highest TAA (418.7 µg/g) while the white baby carrot showed the lowest TAA (3.2 µg/g), With respect to the different proceeding techniques, juices obtained from the low-speed juicer showed the highest amounts of the TAA compared to the high-speed centrifugal juicer and blender, which generated heat during the processing and cause deterioration of the ascorbic acid. Similar results have also been reported

by previous literature (Pyo, Jin, & Hwang, 2014), which reported that the influence of juicing had an overall positive effect on the content on specific fruits. It is therefore, apparent that the low-speed juicer had potential to retain the ascorbic acid of those vegetables. However, three vegetables, green cauliflower, green radish, and organic red kale, their blended juices exhibited the higher TAA than the juices obtained by the other two processing methods. Similar results have also been reported by previous literature (Pyo, Jin, & Hwang, 2014), which reported that the influence of juicing and blending on ascorbic acid content was crop-dependent wherein juicing had an overall positive effect on the content ascorbic acid.

Significant variations were found in nitrate contents of the processed juices, with red beet showed the greatest nitrate value (1270.7 μg/g) while orange baby carrot shows the least nitrate contents. Different from TAA, the effect of processing techniques on nitrate content depended on each vegetable. According to cauliflower, kale, turnip, and beet, their blended juices had higher nitrate content compared to the juices acquired from high-speed centrifugal juicer and low-speed juicer. In comparison, the carrot juices obtained by high- or low-speed juicers exhibited higher nitrate.

3.2.3. Influence of household processing techniques on total phenolics

Phenolic compounds are the secondary metabolites widely present in fruits and vegetables and are among the most desirable food bioactives due to their health-promoting potentials (Naczk & Shahidi, 2006). In vegetables, flavonoids are present as conjugates in glycosylated or esterified forms (Liu, 2004). As a result of processing, these compounds convert to metabolites, especially to their aglycone forms (Cartea, Francisco, Soengas, & Velasco, 2010). The total phenolics (TP) of processed juices are shown in Fig 1A that ranged from 40.33 to 717.67 μg GAE/g FW with the

highest and the lowest value detected in purple baby carrot blended juice and white cauliflower high-speed centrifugal juice, respectively. The kale, radish, and beet juices obtained by low-speed juicing had significantly higher TP than the juices acquired from blending and high-speed centrifugal juicing. This difference could be attributed to the chemical reactions that occurs of the phenolic compounds during the processing. Kale, radish, and beet are mainly composed of kaempferol and quercetin glycosides, cyanidins, and betalains, respectively (Lin & Harnly, 2010; Vulić, et al., 2012). Thermal processing such as blending or high-speed centrifugal juicing generates heat and causes glycosylation, and degrades these compounds, thus reducing the content of total phenolics (Nayak, Liu, & Tang, 2015). In comparison, the low-speed juice extractor squeezes juice with a horizontal auger that rotates food samples at a low speed of 80 rpm, had less effects to those compounds when compared to the blending and high-speed centrifugal juicing methods, since it generated less heat. In comparison, the blended cauliflower, turnip, and baby carrot juices had higher TP than the juices obtained by high-speed centrifugal juicer and low-speed juice extractor. Previous literature reported that the total phenolics differ on the basis of vegetable parts used in the assay and the highest amount was found in peels (D. Zhang & Hamauzu, 2004). The juicing process released all pulps and peels of vegetables that had larger quantities of phenolic compounds (T. Kujala, Loponen, & Pihlaja, 2001), whereas blending retained the pulps and peels. Therefore, turnip and carrot blended juices were significantly higher in total phenolics compared to the other two methods,

Accumulation of phenolic compounds is also differentiated by the color of each vegetable cultivars. In our study, the purple baby carrots contained most phenolic compounds compared to orange, yellow, and white carrots, with the amount 7 fold, 3 fold, and 4 fold in blended juices, for example, as higher than the three cultivars. In case of purple carrots, the presence of phenolic

compounds along with anthocyanins and polyphenolic compounds such as chlorogenic acid, caffeic, and others in a clearly higher concentration than orange, white and yellow carrots, also contributed to the total phenolics (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001). Hence, total phenolics were greatly influenced by genotypes, colors, and processing techniques.

3.2.4. Identification of bioactive compounds in 21 various vegetables processed by three juicing techniques by UHPLC–QTOF-MS analysis

Methanol extracts of 21 vegetables were processed by the three juicing techniques and analyzed using LC-MS. According to the result (Table 1), 72 phytochemical compounds, including flavonols and their glycosides, anthocyanins, 36 betalains, 2 amino acids, and 2 phenolic acids were identified by UHPLC–QTOF-MS analysis (Table 1). These identified compounds were confirmed based on their retention time, accurate mass, possible fragmentation patterns from published papers (Lin & Harnly, 2010; Yang, Jayaprakasha, & Patil, 2018a). Kale extracts exhibited the highest varieties of nonacylated glycosides including kaempferol (m/z 287) and quercetin (m/z 303) glycosides as the main flavonoids (Table 1). The varieties were quercetin-3-diglucoside-7-glucoside and isomer (m/z 789), quercetin-3-sophoroside-7-diglucoside (m/z 951), kaempferol-3-O-diglucoside-7-glucoside (m/z 773), kaempferol-3-O-(caffeoyl)-sophoroside-7-O-glucoside and isomer (m/z 935), kaempferol-3-hydroxyferuloyl-diglucoside-7-glucoside (m/z 965), kaempferol-3-hydroxyferuloyl-diglucoside-7-diglucoside (m/z 1127), kaempferol-3-O-(caffeoyl)-sophoroside-7-O-diglucoside (m/z 1097), quercetin-3-sinapoyl-diglucoside-7-diglucoside (m/z 995), quercetin-3-O-(feruloyl)-sophoroside (m/z 965), kaempferol-3-sinapoyl-diglucoside-7-diglucoside (m/z 1141), kaempferol-3-sinapoyl-diglucoside-7-glucoside (m/z 979), kaempferol-3-feruloyl-diglucoside-7-diglucoside (m/z 1111), kaempferol-3-(feruoyl)-

sophoroside-7-O-glucoside (m/z 949), kaempferol-3-O-sophoroside and isomer (m/z 611), kaempferol-3-O-sophoroside-7-glucoside (m/z 773), kaempferol-3-O-(sinapoyl)-sophoroside (m/z 817), kaempferol-3-O-(feruloyl)-sophoroside (m/z 787), and kaempferol-3-O-glucoside (m/z 449). Previous study has also identified kaempferol and quercetin as the main phenolic compounds in different *Brassica* crops such as kale and cauliflower, in which 3-O-sophoroside-7-O-glucoside and its conjugations with hydroxycinnamic acids, were the main phenolics (Cartea, Francisco, Soengas, & Velasco, 2011). A large quantity of hydroxycinnamic acids; gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic, p-coumaric, caffeic, ferulic, chlorogenic and sinapic acids were detected in leafy *Brassica* vegetables. In our study, only ferulic acid derivative (m/z 325), 5-p-coumaroylquinic acid (m/z 339), and 4-p-coumaroylquinic acid (m/z 339) were identified in kale, cauliflower, turnip, and radish samples. In other *Brassica v*egetables, including all colored cauliflower, turnip, and radish, several phytochemical compounds were identified.

Beet (*Beta vulgaris*) proved to have various betalain compounds which largely contribute to the onset of several degenerative diseases (Slatnar, Stampar, Veberic, & Jakopic, 2015a). Betalains are categorized as red betacyanins and yellow betaxanthins and their levels are substantially influenced by the production systems and cultivars (Stintzing, Schieber, & Carle, 2002a). Our study used conventionally grown (red and golden colored), and organically grown (red and golden colored) beet extracts to unravel the composition differences among the four beet varieties. Thirty-six metabolites include 13 betacyanins, 20 betaxanthins, and 3 amino acids, were tentatively identified. Betanin (m/z 551) and its C-15 isoform isobetanin (m/z 551) with the fragmentation ion at m/z 389 (betanidin) were found to be the main betacyanins in red beet extracts. Other minor betacyanins were mainly presented and protonated ion at m/z 631, 727, and 833, and their ion fragments at m/z at 551 (betanin) and 389 (betanin). The mass differences

between these betacyanins and betanin proposed the existence of extra pentose moiety. This finding is in agreement with the previous study (Stintzing, Schieber, & Carle, 2002a), which verified that betalain concentration differs among beet cultivars, and yellow colored beetroot completely lacked betacyanins. The novel finding of this study asparagine-betaxanthin (m/z 326), threonine-betaxanthin (m/z 313), alanine-betaxanthin (m/z 283), and methionine-betaxanthin (m/z 343), which were identified in beet samples for the first time when, compared to the beet samples from distinct origins or countries (Nemzer, Pietrzkowski, Spórna, Stalica, Thresher, Michałowski, et al., 2011; Slatnar, Stampar, Veberic, & Jakopic, 2015a). Thus, these four uniquely identified betaxanthin compounds may be potentially used as markers to characterize and compare beetroots grown at different geographical locations.

3.2.5. Effect of household processing techniques on DPPH and ABTS radical scavenging activities

Many assays investigating antioxidant activity of vegetables have been developed and applied, however, due to the complex nature of biological systems, it is has been difficult to establish a single universal method, thus, at least two assays should be applied to provide an comprehensive overview of antioxidant activities in one experiment (Šamec, Maretić, Lugarić, Mešić, Salopek-Sondi, & Duralija, 2016). In our study, DPPH and ABTS radical scavenging assays were performed and the activity of vegetable juices obtained by three processing techniques; blending, high-speed centrifugal juicing, and low-speed juice extracting, are given in Fig. 2. Among the 21 vegetables, the highest ABTS activity was found in organic red beetroot processed by high-speed centrifugal juicing (440.97 μg AA/g FW), followed by purple baby carrot blended juice (373.74 AA/g FW), and the lowest ABTS activity was depicted in white cauliflower

blended juice (40.27 μg AA/g FW). The highest and lowest DPPH activities were detected in purple baby carrot juice processed by high-speed centrifugal juicer (715.77 μg AA/g FW), and in yellow baby carrot processed by low-speed juicing (46.46 μg AA/g FW), respectively. The major anthocyanin in purple carrot is cyanidin-3-(sinapoylxylosyl-glucosylgalactoside), which exhibited significant antioxidant activity due to the free hydroxyl groups on the 3´ and 4´ positions, which may have contributed to its high DPPH and ABTS activities of this study (H. Wang, Cao, & Prior, 1997).

Significant variations were found among the vegetables processed by the three techniques. To sum, significant higher ABTS free radical scavenging values were observed in the three colored cauliflowers, organic black kale, turnips, and all baby carrot juices or pastes obtained by blender, compared to the juices obtained from the other two methods. Interestingly, contrasting results were found for green, green organic, red organic kale, four varieties of radishes, golden, and organic golden beetroot processed juices, whereas low-speed juicing showed higher ABTS values. The only exception was organic red beetroot obtained from high-speed centrifugal juicing that showed significantly higher ABTS activity compared to the other techniques. A similar trend was found in DPPH results, where higher values were found in cauliflower, organic black kale, and the four baby carrots juices processed by the blending. While in green kale (conventional and organic), organic red kale, radish, and golden beetroot (conventional and organic), low-speed juicer produced juices showed higher DPPH scavenging activities. Different from ABTS results, turnip juices obtained from high-speed centrifugal juicer had the highest DPPH values. Disparities of these results could be attributed to their initiating mechanisms of the two assays, when either hydrogen or single electron transfer was the dominating reaction (Schaich, Tian, & Xie, 2015).

3.2.6. Inhibition of α-amylase enzymes

The inhibition of α-amylase is necessary as it plays an important role in decreasing postprandial hyperglycemia (Heo, Hwang, Choi, Han, Kim, & Jeon, 2009). In this study, the results of α-amylase inhibition percentage in processed vegetables are shown in Fig 1B. The blended vegetable juices had higher α-amylase inhibition rates when compared to the other two juiced products which can be explained by the retention of high amount of dietary fibers by blending. Dietary fibers from vegetables have proved to have effective anti-diabetic activities (P. Y. Wang, Fang, Gao, Zhang, & Xie, 2016). The highest α-amylase inhibition percentage was found in organic red radish blended juice, with the value of 99.3%. Previous study found that the inhibitory effects of flavonoids were positively correlated with number of hydroxyl groups of the polyphenol ligands and formation of a conjugated-systems which stabilized the interaction on the active site (Sales, Souza, Simeoni, Magalhães, & Silveira, 2012). *Brassica* vegetables were rich in flavonoids; thus, their α-amylase inhibition activities were potent. The lowest α-amylase inhibition percentage was detected in yellow cauliflower juice obtained by high-speed centrifugal juicing, with the value of 57.41%. Organic golden beetroot juice obtained by low-speed juice extractor also had low inhibition percentage of 60.08%. The remaining vegetables juices showed high α-amylase inhibition percentage, with the values ranging from 70.47% to 96.96%.

3.2.7. Chemometrics-based analysis in discriminating phytochemicals profiles under three processing techniques

The chemometric-based analysis was carried out based on the LC-MS data to visualize the characteristic components in the processed juice samples. The unsupervised principal component analysis (PCA) approach was not able to achieve the separation of the three processing techniques;

blending, high-speed centrifugal juicing, and low-speed juicing (Appendix Fig.1). This was due to the presence of similar compounds in the processed samples that only differs on the basis of peak intensities. In the next step, we performed partial least squares discriminant analysis (PLS-DA), the supervised pattern recognition platform to discriminate the phytochemical profiles in processed vegetables under the three techniques. With respect to *Braccica* vegetable juices, our study identified 20 metabolites, mainly kaempferol glycosides and phenolic acids, and are considered to be important for the differentiation of the blending, high-speed centrifugal juicing, and low-speed juicing samples. The levels of all the metabolites were highest in low-speed kale juices when compared to juices obtained by blending or high-speed centrifugal juicing (Fig.3). Based on the heatmap, the low-speed juicing retained more varieties of metabolites compared to the other techniques and a large percentage of kaempferol and quercetin glycosides were detected in abundance, indicating the potential development of nonthermal process techniques. It is worthy to mention that the processing techniques and vegetable varieties had effect on composition, amongst the green kale blended juices shows the presence of less metabolites than the high- or low-speed juices. However, this difference was not exhibited when the metabolites composition of black kale juices was compared from the three processing techniques. In order to find the metabolites that make the most significant contributions to classification, variable importance for projection (VIP) score plot (Appendix Fig.1) was generated to provide an overview of the levels of metabolites were selected by specific VIP values (>1) with the results indicated that the low-speed juicing has higher levels of these compounds.

The impact of processing techniques on phytochemical profiles in four varieties of beet were also analyzed by the metabolomics combined with chemometrics and the results are shown in Fig.3. Unlike kale samples whose major metabolites were generally higher in the low-speed

juicer compared to the other two methods, the levels of each metabolite varied in beet samples. In general, the blended conventional red beet samples retained higher amounts of isobetanin (m/z 551), neobtanin (m/z 549), prebetanin (m/z 631), 6'feruloyl-betanin and its isomer (m/z 727) but similar levels of betaxanthins compared to high-speed centrifugal juicer and low-speed juicer. The VIP score plot (Appendix Fig.1) revealed four characteristic betalain compounds, in which the glutamine-betaxanthin (m/z 340) and isoleucine-betaxanthin (m/z 325) were the highest in blended juices, and dopamine-betaxanthin (m/z 347) were the highest in low-speed juicer, and neobetanin (m/z 549) was the highest in high-speed centrifugal juicer, respectively.

3.3. Conclusions

Our study indicated that the three processing techniques; blending, high-speed centrifugal juicing, and low-speed juicing, substantially affected the compositions and antioxidant activities of the commonly consumed vegetables. 73 phytochemicals (36 betalains, 23 flavonols, 6 anthocyanidins, 6 phenolic acids and 2 amino acids) including four novel betalains (asparagine-betaxanthin, threonine-betaxanthin, alanine-betaxanthin, and methionine-betaxanthin), were identified by the untargeted UPLC-HR-ESI-QTOF-MS profiling technique. The targeted metabolomics approach combined with chemometrics revealed that several kaempferol glycosides and amino acid adducted betaxanthins were the potential key metabolites to discriminate the different processed kale and beet juices that varied among vegetable varieties and the processing techniques. In addition, the color attributes, phytochemical contents, and antioxidant activities of each vegetable were also significantly influenced by the three processing techniques. This study demonstrated that the metabolomics-based approach combined with chemometrics provides a fast and efficient tool to screen the impact of the processing techniques by highlighting the metabolomic differences of the commonly consumed vegetables.

Table 1. Identification of bioactive compounds from 21 processed vegetables using UPLC-HR-ESI-QTPF-MS.

RT (Min)	Identified compounds	Molecular formula	Experimental mass	Exact mass	Mass error (ppm)	MS/MS fragments	Occurrence			Reference
Flavonols and their derivatives										
							Brasicca	*Beta vulgaris*	*D. Carota*	
2.45	Epigallocatechin	$C_{15}H_{14}O_7$	307.0953	307.0812	45.9	-	x			
8.05	Quercetin-3-diglucoside-7-glucoside	$C_{30}H_{40}O_{22}$	789.2230	789.2084	18.5	541, 465, 303, 237, 177	x			(Yang, Jayaprakasha, & Patil, 2018a)
8.15	Quercetin-3-diglucoside-7-glucoside (isomer)	$C_{30}H_{40}O_{22}$	789.2238	789.2084	19.5	571, 478, 409, 303, 163	x			
8.35	Quercetin-3-sophoroside-7-diglucoside	$C_{39}H_{50}O_{27}$	951.2772	951.2612	16.8	571, 449, 303, 237, 153	x			
8.50	Kaempferol-3-O-diglucoside-7-glucoside	$C_{33}H_{40}O_{22}$	773.2263	773.2135	16.6	633, 541, 449, 347, 287, 177	x			
8.70	Kaempferol-3-O-(caffeoyl)-sophoroside-7-O-glucoside	$C_{42}H_{46}O_{24}$	935.2800	935.2452	37.2	571, 287, 193, 85	x			
8.95	Kaempferol-3-hydroxyferuloyl-diglucoside-7-glucoside	$C_{43}H_{48}O_{25}$	965.2721	965.2557	17.0	539, 492, 355, 287, 193, 133	x			
9.10	Kaempferol-3-hydroxyferuloyl-diglucoside-7-diglucoside	$C_{49}H_{58}O_{30}$	1127.3283	1127.3086	17.5	539, 449, 355, 287, 193	x			
9.20	Kaempferol-3-O-(caffeoyl)-sophoroside-7-O-glucoside (isomer)	$C_{42}H_{46}O_{24}$	935.2632	935.2452	19.2	409, 287, 163, 84	x			
9.35	Kaempferol-3-O-(caffeoyl)-sophoroside-7-O-diglucoside	$C_{45}H_{60}O_{31}$	1097.3190	1097.3191	-0.1	531, 287, 207, 163	x			
9.45	Quercetin-3-sinapoyl-diglucoside-7-diglucoside	$C_{50}H_{60}O_{31}$	995.2880	995.2663	21.8	553, 409, 303, 207	x			
9.55	Quercetin-3-O-(feruloyl)-sophoroside	$C_{43}H_{48}O_{25}$	965.2792	965.2557	24.3	523, 409, 339, 303, 177	x			
9.85	Kaempferol-3-sinapoyl-diglucoside-7-diglucoside	$C_{50}H_{60}O_{30}$	1141.3535	1141.3242	25.7	553, 369, 287, 207	x			
10.00	Kaempferol-3-sinapoyl-diglucoside-7-glucoside	$C_{44}H_{50}O_{25}$	979.3011	979.2714	30.3	707, 501, 369, 287, 177	x			
10.05	Kaempferol-3-feruloyl-diglucoside-7-diglucoside	$C_{49}H_{58}O_{29}$	1111.3503	1111.3137	32.9	707, 523, 449, 339, 287, 177	x			
10.20	Kaempferol-3-(feruloyl)-sophoroside-7-O-glucoside	$C_{43}H_{48}O_{24}$	949.2491	949.2608	-12.3	809. 624, 523, 449, 339, 287, 177	x			
11.25	Kaempferol-3-O-sophoroside	$C_{27}H_{30}O_{16}$	611.1536	611.1607	-11.6	553, 462, 329, 287, 177	x			

Table 1. Continued

RT (Min)	Identified compounds	Molecular formula	Experimental mass	Exact mass	Mass error (ppm)	MS/MS fragments	Occurrence			Reference
Flavonols and their derivatives										
							Brasicca	*Beta vulgaris*	*D. Carota*	
12.00	Kaempferol-3-O-sophoroside (isomer)	$C_{27}H_{30}O_{16}$	611.1540	611.1607	-11.0	427, 347, 287, 177	x			(Yang, Jayaprakasha, & Patil, 2018a)
13.75	Kaempferol-3-O-sophoroside (isomer)	$C_{27}H_{30}O_{16}$	611.1539	611.1607	-11.1	519, 369, 287, 207, 175	x			
13.95	Kaempferol-3-O-glucoside	$C_{21}H_{20}O_{11}$	449.1029	449.1078	-10.9	395, 287, 177, 91	x			
15.00	Disinapoyl-diglucoside	$C_{34}H_{42}O_{19}$	777.2139	777.2213	-9.5	665, 553, 479, 347, 287, 207, 91	x			
15.40	1-Sinapoyl-2-feruloyl-diglucoside	$C_{33}H_{40}O_{18}$	747.2272	747.2107	22.1	523, 339, 287, 207, 177, 145	x			
16.70	Trisinapoyl-diglucoside	$C_{45}H_{52}O_{23}$	983.2661	983.2797	-13.8	682, 523, 369, 287, 207, 177	x			
Anthocyandins Devivatives										
2.10	Cyanidin	$C_{15}H_{11}O_6^+$	288.2081				x			(Algarra, Fernandes, Mateus, de Freitas, da Silva, & Casado, 2014)
8.65	Cyanidin 3-xylosyl-galactoside	$C_{26}H_{29}O_{15}^+$	581.1564	581.1000		479, 377, 287, 163				
8.85	Cyanidin 3-xylosyl-galactoside (isomer)	$C_{26}H_{29}O_{15}^+$	581.1559	581.1000		479, 377, 287, 163				
9.55	Cyanidin 3-xylosyl (sinapoylglucosyl)galactoside	$C_{43}H_{49}O_{24}^+$	949.2667	949.2608	6.2	673, 287				
10.00	Cyanidin 3-xylosyl(feruloylglucosyl)-galactoside	$C_{42}H_{47}O_{23}^+$	919.2607	919.2503	11.3	823, 655, 575, 411, 365, 287				
10.80	Cyanidin 3-xylosyl(feruloylglucosyl)-galactoside (isomer)	$C_{42}H_{47}O_{23}^+$	919.2616	919.2503	12.3	823, 655, 575, 411, 365, 287				

Table 1. Continued

RT (Min)	Identified compounds	Molecular formula	Experimental mass	Exact mass	Mass error (ppm)	MS/MS fragments	Occurrence			Reference
Betalains Derivatives										
							Brasicca	*Beta vulgaris*	*D. Carota*	
1.00	Asparagine-betaxanthin (Vulgaxanthin III)	$C_{13}H_{15}N_3O_7$	326.0990	326.0983	2.1	203, 137		x		(Nemzer, et al., 2011)
1.80	Glutamine-betaxanthin	$C_{14}H_{17}N_3O_7$	340.1155	340.1139	4.7	321, 277		x		
2.50	Glutamine-isobetaxanthin	$C_{14}H_{17}N_3O_7$	340.1206	340.1139	-15.0	-		x		
3.20	Threonine-betaxanthin	$C_{13}H_{16}N_2O_7$	313.1033	313.1030	1.0	-		x		
3.80	Glutamic acid-betaxanthin	$C_{14}H_{16}N_2O_8$	341.0987	341.0979	2.3	215		x		
4.05	Glutamic acid-isobetaxanthin	$C_{14}H_{16}N_2O_8$	341.1056	341.0979	22.6	-		x		
4.35	Alanine-betaxanthin	$C_{12}H_{14}N_2O_6$	283.0928	283.0925	1.1	164, 84		x		
4.50	17-Decarboxy-betanidin	$C_{17}H_{17}N_2O_6^+$	345.1084	345.1081	0.9	-		x		
4.55	Valine-isobetaxanthin	$C_{14}H_{18}N_2O_6$	311.1302	311.1238	20.6	-		x		
4.55	2,17-Bidecarboxy-betnidin/isobetanidin	$C_{22}H_{27}N_2O_9^+$	463.1715	463.1711	0.9	242, 164		x		
4.95	γ-Aminobutyric acid-betaxanthin	$C_{13}H_{16}N_2O_6$	297.1084	297.1081	1.0	-		x		
5.05	Proline-betaxanthin	$C_{14}H_{16}N_2O_6$	309.1107	309.1081	8.4	283, 195		x		
5.55	Prebetanin	$C_{24}H_{26}N_2O_{16}S$	631.1067	631.1076	-1.4	551, 389		x		
5.75	Betanin	$C_{24}H_{26}N_2O_{13}$	551.1532	551.1508	4.4	389, 150		x		
5.85	Isobetanin	$C_{24}H_{26}N_2O_{13}$	551.1523	551.1508	2.7	389, 150		x		
6.35	Dopamine-betaxanthin (Miraxanthin V)	$C_{17}H_{18}N_2O_6$	347.1247	347.1238	2.6			x		
6.40	Tyrosine-isobetaxanthin (Isoportulacaxanthin II)	$C_{18}H_{18}N_2O_7$	375.1263	375.1187	20.3			x		
6.45	Methionine-betaxanthin	$C_{14}H_{18}N_2O_6S$	343.0968	343.0958	2.9			x		
6.75	Valine-betaxanthin	$C_{14}H_{18}N_2O_6$	311.1242	311.1238	1.3	-		x		
7.05	Miraxanthin III	$C_{17}H_{18}N_2O_5$	331.1291	331.1288	0.9	121, 84		x		
7.15	17-Decarboxy-neobetanin	$C_{23}H_{24}N_2O_{11}$	505.1459	505.1453	1.2	343, 297		x		
7.30	Neobetanin	$C_{24}H_{24}N_2O_{13}$	549.1368	549.1352	2.9	387		x		
8.15	Isoleucine-isobetaxanthin	$C_{13}H_{15}N_3O_7$	325.1403	325.1394	2.8	-		x		

Table 1. Continued

RT (Min)	Identified compounds	Molecular formula	Experimental mass	Exact mass	Mass error (ppm)	MS/MS fragments	Occurrence			Reference
Betalains Derivatives										
							Brasicca	*Beta vulgaris*	*D. Carota*	
8.30	Isoleucine-betaxanthin	$C_{13}H_{15}N_3O_7$	325.1410	325.1394	4.9	-		x		(Nemzer, et al., 2011)
8.50	Phenylalanine-isobetaxanthin	$C_{18}H_{18}N_2O_6$	359.1261	359.1238	6.4	303, 177		x		
8.60	S-tryptophan-betaxanthin	$C_{20}H_{19}N_3O_6$	398.1348	398.1347	0.3	-		x		
8.80	Leucine-isobetaxanthin (Isovulgaxanthin IV)	$C_{13}H_{15}N_3O_7$	325.1403	325.1394	2.8			x		
8.95	2-Decarboxy-2,3-dehydro-neobetanin	$C_{23}H_{22}N_2O_{11}$	503.1303	503.1296	1.4	341		x		
9.05	17-Decarboxy-2,3-dehydro-neobetanin	$C_{23}H_{22}N_2O_{11}$	503.1298	503.1296	0.4	341		x		
9.15	15-Decarboxy-2,3-dehydro-neobetanin	$C_{23}H_{22}N_2O_{11}$	503.1299	503.1296	0.6	341		x		
9.55	Betanidin	$C_{18}H_{16}N_2O_8$	389.1016	389.0979	9.5	343		x		
9.80	6´-feruloyl-betanin	$C_{34}H_{35}N_2O_{16}^+$	727.1966	727.1981	-2.1	389		x		
9.90	6´-feruloyl-isobetanin	$C_{34}H_{35}N_2O_{16}^+$	727.1993	727.1981	1.7	389		x		
10.05	17-Decarboxy-betanin	$C_{22}H_{26}N_2O_{11}$	507.1606	507.1609	-0.6	345		x		
10.05	15-Decarboxy-betanin	$C_{22}H_{26}N_2O_{11}$	507.1603	507.1609	-1.2	345		x		
Phenolic acids										
0.80	Ferulic acid derivative		325.1229				x	x	x	(Kammerer, Carle, & Schieber, 2004)
6.95	Syringic acid	$C_9H_{10}O_5$	199.0590	199.0601	-5.5	-		x		
7.55	5-P-Coumaroylquinic acid	$C_{16}H_{18}O_8$	339.1035	339.1074	-11.5	-	x			
7.85	4-P-Coumaroylquinic acid	$C_{16}H_{18}O_8$	339.1036	339.1074	-11.2	-	x			
8.30	3-O-Caffeoylquinic acid	$C_{16}H_{18}O_9$	355.1027	355.1024	0.8	-			x	
8.70	5-O-Caffeoylquinic acid	$C_{16}H_{18}O_9$	355.1034	355.1024	2.8	-			x	
Amino acids										
3.45	Phenylalanine	$C_9H_{11}NO_2$	166.0863	166.0863	0.0	-		x	x	(Kammerer, Carle, & Schieber, 2004)
6.25	L-Tryptophan	$C_{11}H_{12}N_2O_2$	205.0947	205.0972	-12.2	-	x	x		(Kammerer, Carle, & Schieber, 2004)

Table 2. Levels of total ascorbic acid (TAA), and nitrate contents of processed vegetable samples.

	Blender		High-speed centrifugal Juicer		Low-speed juicer	
	TAA	Nitrate	TAA	Nitrate	TAA	Nitrate
Brassicaceae						
White cauliflower	19.6 ± 0.6f	32.4 ± 2.4c	34.6 ± 0.9ef	24.6 ± 1.2cd	418.7 ± 20.5a	22.5 ± 0.8cde
Yellow cauliflower	19.3 ± 3.1f	13.4 ± 0.3e	66.8 ± 1.1de	22.2 ± 1.2de	295.9 ± 5.2b	26.1 ± 0.3cd
Green cauliflower	135.0 ± 7.7c	91.4 ± 7.7a	137.4 ± 6.7c	53.9 ± 0.7b	70.7 ± 0.2d	57.5 ± 0.6b
Green kale	80.6 ± 4.5d	13.4 ± 0.6f	125.0 ± 2.4c	60.7 ± 0.7e	111.3 ± 7.1c	7.1 ± 0.3f
Organic green kale	115.5 ± 9.4c	994.5 ± 50.71a	340.0 ± 7.2a	509.0 ± 17.5de	226.1 ± 8.3b	631.3 ± 17.2c
Organic red kale	70.5 ± 9.9d	711.1 ± 26.6b	25.0 ± 0.5e	546.4 ± 8.4d	51.4 ± 1.7de	559.1 ± 3.3d
Purple-white turnip	41.8 ± 0.3d	982.37 ± 6.5a	126.5 ± 0.4c	922.46 ± 21.3b	199.3 ± 1.2b	951.12 ± 14.7ab
White baby turnip	38.2 ± 0.7d	405.55 ± 0.8c	196.8 ± 2.0b	377.67 ± 8.8c	268.6 ± 5.6a	315.03 ± 1.3d
Red radish	50.5 ± 1.3d	578.1 ± 5.5a	174.0 ± 7.1ab	236.9 ± 7.1f	57.0 ± 1.0d	398.5 ± 5.9c
Green radish	153.6 ± 15.5abc	279.9 ± 1.2e	126.9 ± 5.0bc	499.6 ± 8.0b	144.1 ± 15.0abc	315.7 ± 8.9d
White radish	14.0 ± 0.4d	147.2 ± 1.7g	107.1 ± 7.1c	148.1 ± 4.9g	183.5 ± 18.5a	233.5 ± 0.9f
Red Beet	12.3 ± 0.4cde	298.3 ± 19.9bc	12.1 ± 0.8cde	1270.7 ± 19.9a	22.0 ± 1.5ab	52.8 ± 2.2c
Organic red beet	9.0 ± 0.4de	601.2 ± 3.2b	8.7 ± 0.4de	321.7 ± 4.9bc	13.4 ± 0.4cd	175.5 ± 0.6c
Golden beet	6.4 ± 0.3e	381.6 ± 17.3bc	17.7 ± 0.5bc	213.2 ± 0.8bc	25.0 ± 0.4a	275.4 ± 1.6bc
Organic golden beet	12.3 ± 0.2cd	30.3 ± 0.8c	23.3 ± 0.1ab	19.0 ± 0.4c	26.6 ± 3.4a	50.4 ± 0.8c
Orange baby carrot	2.6 ± 0.3f	10.9 ± 0.4g	6.5 ± 0.8e	50.7 ± 1.9f	8.2 ± 0.6de	122.2 ± 0.1d
Purple baby carrot	17.5 ± 0.3c	12.8 ± 1.7g	22.0 ± 0.7b	85.7 ± 0.9e	28.9 ± 0.5a	220.0 ± 2.0b
Yellow baby carrot	7.7 ± 0.6e	170.9 ± 2.2c	11.0 ± 0.6d	305.7 ± 6.4a	7.8 ± 0.5e	86.2 ± 2.1e
White baby carrot	3.2 ± 0.6f	16.1 ± 0.4g	8.3 ± 1.2de	129.6 ± 0.6d	8.1 ± 0.1de	285.5 ± 13.8a

Data are expressed as mean ± SE (n = 3); values within each type of sample marked by the different letter within same column are significantly different ($P < 0.05$). All data were based on fresh weight basis.

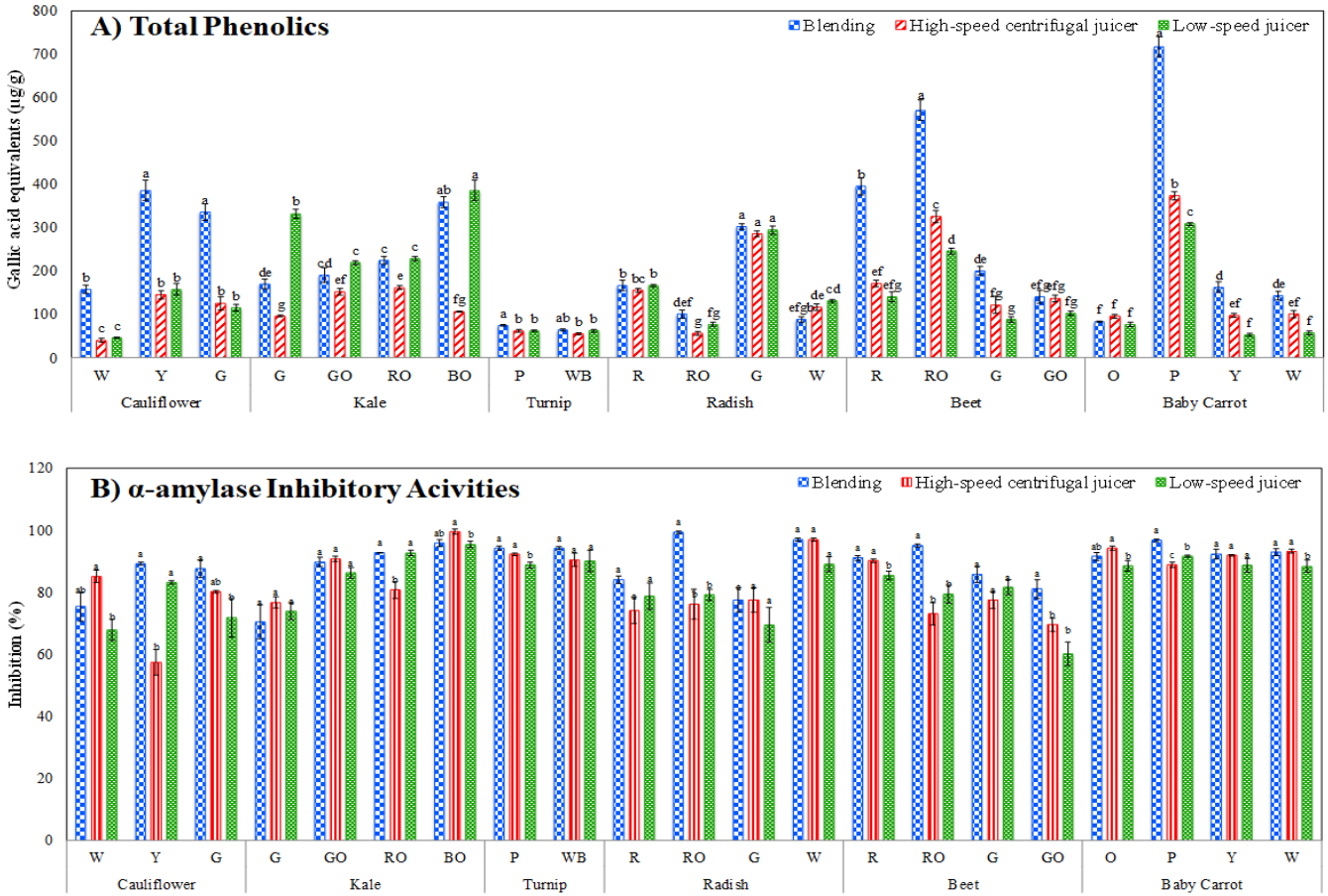

Figure 1. The (A) total phenolics and (B) α-Amylase inhibitory activity of 21 vegetables processed by three techniques. Data are expressed as means ± SE of three replications. Means with same letter indicate no significant difference between treatments ($P<0.05$). W: white, Y: yellow, G: green, GO: green organic, BO: black organic, P: purple, R: red, RO: red organic; G: golden, GO: golden organic.

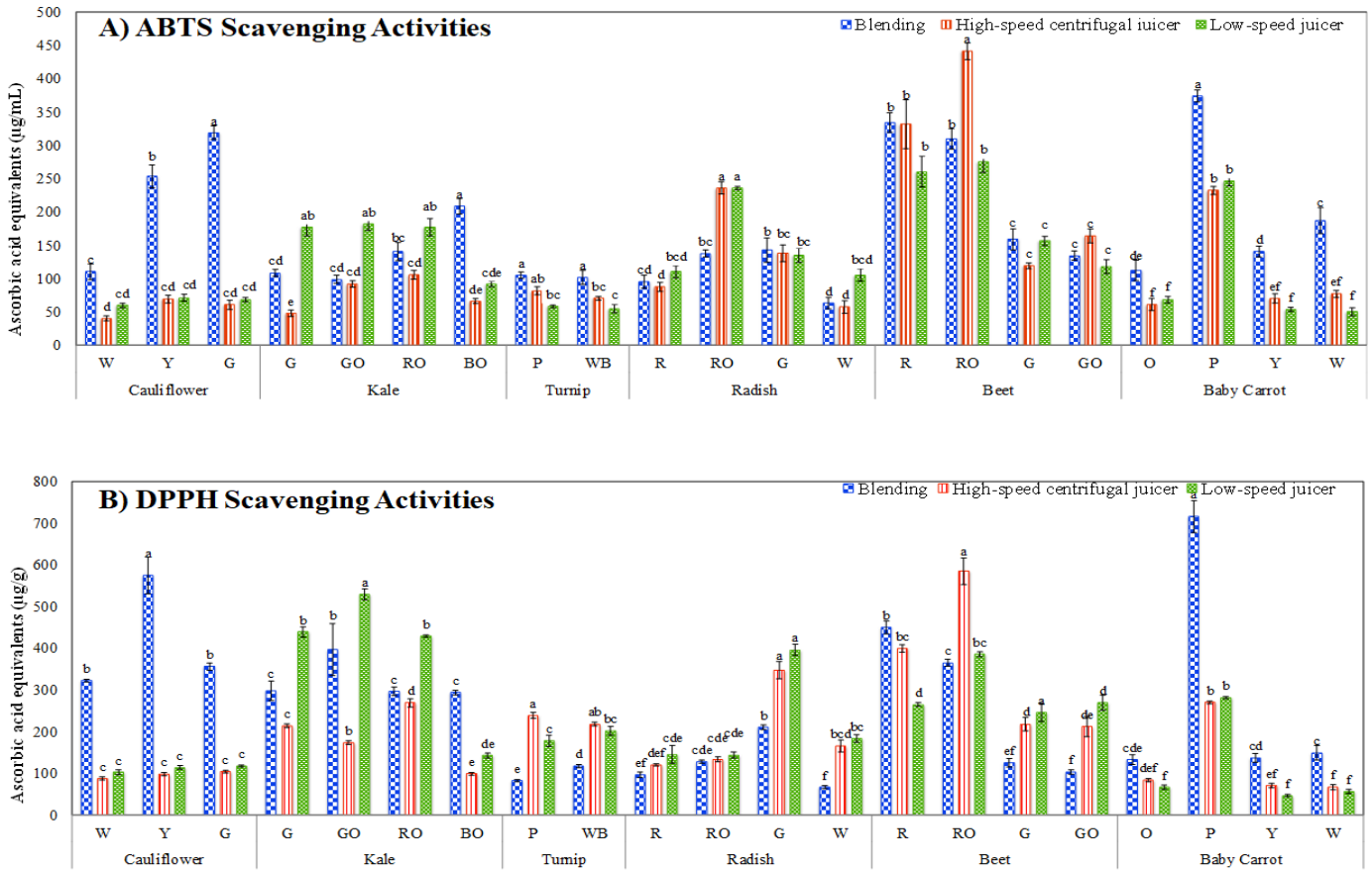

Figure 2. The (A) ABTS and (B) DPPH scavenging activity of 21 vegetables processed by three techniques. Data are expressed as means ± SE of three replications. Means with same letter indicate no significant difference between treatments ($P<0.05$). W: white, Y: yellow, G: green, GO: green organic, BO: black organic, P: purple, R: red, RO: red organic; G: golden, GO: golden organic.

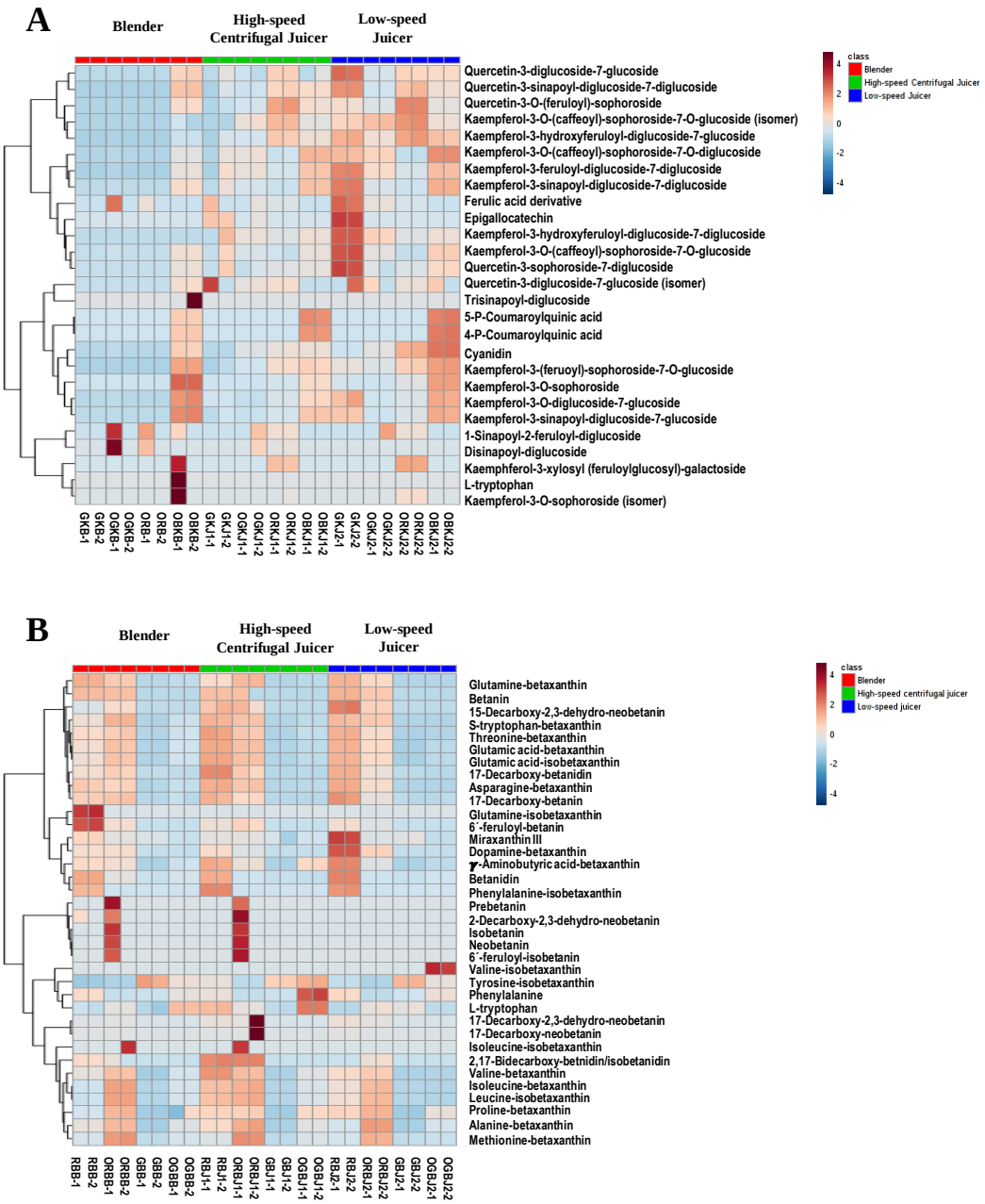

Figure 3. Relative abundance of significantly changed metabolites are presented as box-and-whisker plot in (A) kale and (B) beet processed samples. The color box for each compound indicates the abundance of the compound, with the brown color annotates higher and the blue color represents lower abundance. The *x* and *y* axis represent sample type and compounds names, respectively. Sample types were shown as vegetable name plus processing techniques. Processing techniques were abbreviated as B/J1/J2 representing blending/high-speed centrifugal juicing/low-speed juicing. Kale sample name abbreviations: GK: green kale; OGK: organic green kale; OBK, organic black kale. Beet sample name abbreviations: RB: red beet; ORB: organic red beet; GB: golden beet; OGB: organic golden beet.

4. CHEMOMETRIC CHARACTERIZATION OF 30 COMMERCIAL THERMAL AND COLD PROCESSED JUICES USING UPLC-QTOF-HR-MS FINGERPRINTS

Epidemiological studies have shown that fruits and vegetables are desirable sources of diverse health-promoting compounds with the potential to decrease the occurrence of oxidative stress-related diseases including cardiovascular disease, stroke, diabetes, Alzheimer's disease, and neurodegenerative diseases (Hung, Joshipura, Jiang, Hu, Hunter, Smith-Warner, et al., 2004). These various health-promoting compounds (ascorbic acid, phenolics, betalains, and polyphenols) quench the proliferation of radical oxygen (ROS) and reaction nitrogen (RNS) species, which are implicated in these diseases (D. Huang, Ou, & Prior, 2005).

Kale (*Brassica oleracea*) and beetroot (*Beta vulgaris*) are regarded as functional foods based on their putative health-promoting properties, but their tastes are not acceptable to certain consumers (Damunupola, Weerathilake, & Sumanasekara, 2014; S. Y. Kim, Sun, Kwon, Park, & Lee-Kim, 2008). Moreover, many consumers find it inconvenient to consume large melons or prepare fresh vegetables. Therefore, in recent years, food manufacturers have focused on producing beverages with a multitude of functional ingredients to meet consumers' need for convenience, palatability, and wellbeing.

To preserve the health-promoting properties of the functional foods in these beverages, numerous processing strategies have been used to obtain beverages with a refreshing taste, optimal nutrients, and few or no active microbes. Among the techniques used to process commercial juices, thermal processing is commonly used in the production of fruit and vegetable beverages due to its convenience and low cost (Jiménez-Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017). Thermal processing transfers heat from the fastest processing medium to the slowest heating zone of the food, which causes detrimental effects on physical texture, color,

organoleptic qualities, and nutritional values, leading to consumer dissatisfaction with the resulting beverage (C.-Y. Wang, Huang, Hsu, & Yang, 2016).

Recent consumer demand for safer and more natural processed food has induced researchers and manufacturers to develop products that undergo minimal processing (Bhat & Stamminger, 2015). Non-thermal processing technologies have been widely implemented and produce food products with desirable sensory characteristics, improved nutritional values, and enhanced health-promoting functionalities (Barba, Mariutti, Bragagnolo, Mercadante, Barbosa-Cánovas, & Orlien, 2017b). In comparison to other non-thermal processing technologies (pulsed electric field, pulsed light, electron beam, plasma, etc), high-pressure processing (HPP) has been approved in United States and is one of the most successfully commercialized and promising non-thermal techniques used worldwide (H.-W. Huang, Wu, Lu, Shyu, & Wang, 2017). In HPP, food materials were treated with static high pressure for an appropriate period to preserve the flavor, color, and to extend shelf life by inactivating pathogenic microorganisms. HPP has proven to be an effective processing technology for liquid products with heat sensitivity, high-acid juices, blends, smoothies, fermented and fortified fruit and vegetable products, and processed beverages (C.-Y. Wang, Huang, Hsu, & Yang, 2016).

Despite the advantages of the HPP technologies, consumers are still concerned with the authenticity of the ingredients of commercial juices, since they may not match the nutritional label claims (Borges, Mullen, & Crozier, 2010). Additionally, the price of commercially available non-thermal processed juice (example: cold-pressed juice) tends to be 5-fold higher than traditionally pasteurized juices (Table A-2). Previous studies have investigated the effect of HPP on bioactive compounds and antioxidant activities in fruits and vegetables, but a comprehensive study comparing the effects of thermal processing and HPP on phytochemical compounds and

antioxidant activities in commercial fruit and vegetable juices were not studied systematically due to the difficulty in screening the complicated chemical system in each juice.

Chemometrics is an efficient tool to classify products based on their chemical composition. In the area of food and beverages, numerous investigations have been performed to discriminate origin, assess authenticity, and evaluate quality, by chemometrics coupled with analytical techniques such as mass spectrometry (MS) coupled to LC, GC, and nuclear magnetic resonance (NMR), which is an effective tool which allows the characterization of the sensitive plant compounds and is able to identify active constituents with low abundance (Fidelis, Santos, Coelho, Rodionova, Pomerantsev, & Granato, 2017; Jandrić, Roberts, Rathor, Abrahim, Islam, & Cannavan, 2014). Next, in order to process and interpret complex data obtained within metabolomic-based studies, advanced data processing software algorithms and multivariate chemometric tools are needed.

In the present study, for the first time, the use of UPLC-QTOF-HR-MS fingerprints combined with multivariate data analysis were employed for classification of commercial fruit and vegetable juices with different processing techniques and prices. Highly complex HPLC-MS records were subjected to chemometric partial least square discriminant analysis (PLS-DA) analysis and heatmap to differentiate and identify unique health-promoting compounds. In addition, the variations in physicochemical attributes (color, Brix, and pH), phytochemical profiles (ascorbic acid, total ascorbic acid, total phenolics), and radical-scavenging activities (DPPH and ABTS) of these juices were also investigated.

4.1. Materials and methods

4.1.1. Juice samples

Twenty-seven fruit juices were collected from local retailers such as HEB, Kroger, Walmart, and Farm Patch, based on their price, ingredients, and processing types (Table A-2). In addition, six melon juices (M5-M10) were obtained from Savor Fresh Farms (Yuma, AZ). According to their ingredients, juices were divided into three major groups, which include kale, beetroot, and melon juices. Among the 30 juices, 13 were processed by thermal processing, and 17 were processed by cold-pressing combined with high-pressure processing (HPP).

4.1.2. Chemicals and reagents

L-ascorbic acid, gallic acid, sodium carbonate, 2,2-diphenyl-1-picryhydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), Folin-Ciocalteu reagent, HPLC grade methanol, and meta phosphoric acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Nitrate standards were purchased from Alfa Aesar (Wars Hill, MA, U.S.A.). HPLC-grade water (resistivity 18.2 mΩ cm) was obtained from a Nanopure water purification system (Barnstead, Dubuque, IA).

4.1.3. Color measurements

Lightness (L*), a* (green to red), b* (blue to yellow), and C (Chroma) were measured using a Minolta CR-200 Chroma Meter (Minolta, Osaka, Japan). The instrument was calibrated by a white tile standard. Hue was calculated by the equation $H° = \tan^{-1} (b^*/ a^*)$, where H° is the attribute which defines how the specific color is categorized.

4.1.4. pH and soluble solid measurements

The pH values were measured at room temperature with a pH meter (Mettler Toledo, OH, U.S.A.). Total soluble solids contents were estimated with a portable refractometer (Reichert, NY, U.S.A.). For each juice sample, the value was taken as the average of six measured results.

4.1.5. Determination of ascorbic acid, total ascorbic acid, and nitrate in commercial juices

Ascorbic acid (AA) and total ascorbic acid (TAA) were analyzed based on our published paper (Chebrolu, Jayaprakasha, Yoo, Jifon, & Patil, 2012b). AA and TAA were quantified by an Agilent 1220 Series HPLC System, equipped with an Eclipse Plus C18 column (250 mm × 4.6 mm, 5 μm) and a photodiode array detector, was used with isocratic elution using 0.03 M aqueous phosphoric acid as a mobile phase. A 10 μL sample was injected into the column with a flow rate of 1 mL/min, and the peaks were monitored at 244 nm with a run time of 18 min.

Nitrate determination was based on our previously published protocol (Corleto, Singh, Jayaprakasha, & Patil, 2018) with slight modifications. An aliquot of 5 μL was injected into an Agilent 1200 Series HPLC System equipped with an Eclipse Plus C18 column (250 mm × 4.6 mm, 5 μm) and a photodiode array detector. The mobile phase consisted of an isocratic elution of 0.03 M aqueous phosphoric acid, as a mobile phase with a flow rate of 0.5 mL/min, was used. Chromatograms were acquired at wavelength of 210 nm. The total run time was 15 min. Results were expressed as μg of nitrate per mL of fresh juice.

4.1.6. Sample preparation for total phenolics and antioxidant activities

Five milliliters of each juice sample were extracted with 10 mL of methanol by vortexing for 30 s and homogenization for 1 min. After the samples were centrifuged at 4480 g for 10 min,

the supernatant was measured. The residue was re-extracted by adding 3 mL methanol as mentioned above and the supernatant from both extractions was pooled, then the final volume was measured.

4.1.6.1. Total phenolics

Total phenolics were estimated by the Folin-Ciocalteu method as described by Chaudlhary et al (Chaudhary, Jayaprakasha, Porat, & Patil, 2012). The absorbance was monitored at 760 nm with a microplate reader (Bio Tek Instruments, Winooski, VT). Total phenolics were expressed as gallic acid equivalents in µg /mL of juice sample.

4.1.6.2. DPPH free radical scavenging activity

The DPPH free radical scavenging activity was performed according to our published protocols (Chaudhary, Jayaprakasha, Porat, & Patil, 2012) and results were expressed as µg of ascorbic acid equivalents per mL of juice sample.

4.1.6.3. ABTS free radical scavenging activity

The ABTS cation radical eliminating activity was determined according to the method outlined by Jayaprakasha and others (G. K. Jayaprakasha, Girennavar, & Patil, 2008). The reaction was initiated by adding 180 µL ABTS stock solution into the mixture and the absorbance was recorded at a wavelength of 734 nm. Results were expressed as ascorbic acid equivalents µg per mL of fresh juice.

4.1.7. Identification of phenolics by UPLC-HR-ESI-QTOF-MS

UPLC-HR-ESI-QTOF-MS was used for the identification of phenolic compounds in the 30 juice samples. The separation was performed on a 1290 Agilent HPLC LC system (Agilent Technologies, Santa Clara, CA) equipped with a maXis impact mass spectrometer (Bruker Daltonics, Billerica, MA, USA). A rapid resolution Eclipse plus C_{18} column (1.8 μm, 50 × 2.1 mm; Agilent) was used for separation of phenolic compounds with the binary mobile phase consisting of (A), 0.2% formic acid in water, and (B), 0.2% formic acid in water: 0.2% formic acid in acetonitrile (7:3). The column temperature was set at 35°C. The injection volume was 2 μL, the flow rate was 0.3 mL/min, and the runs were monitored at wavelengths of 280 and 320 nm. The following gradient was applied: 100% A (0–2 min), 100–20% A, 0–80% B (2–15 min), and 100–0% B (19–20 min). The high-resolution mass spectral data were obtained by electrospray positive ionization. MS and the broadband collision-induced dissociation (bbCID) data were acquired at a m/z range of 50–1000 using electrospray ionization (ESI) mode. The capillary voltage of the ion source was 4,200 V. The nebulizer gas pressure was 2.8 bar and the drying gas flow rate 8.0 L/min. Nitrogen was used for both nebulizer and drying gas. The drying gas temperature was 220°C. The transfer time of the source was 120.8 μs and the prepulse storage time was 1 μs. The quadrupole MS collision energy and bbCID collision energy were set at 5 and 20 eV, respectively. The spectra rate was 1.4 Hz. For bbCID, the precursor ions were fragmented in the collision cell without pre-selection. By alternating the acquisition between MS and bbCID conditions, high and low collision energy data sets were collected simultaneously. External instrument calibration was performed with sodium formate. Nine sodium formate clusters were used in the calibration in high-precision calibration mode. An automated post-run internal mass scale calibration of individual samples was performed by injecting the above calibrant at the beginning and end of each run. The accurate mass

data were analyzed using the Data Analysis 4.3 software. The molecular formula for each compound was found by mass accuracy, isotopic patterns, adduct and fragment information using SmartFormula. Furthermore, the mass data were exported to Bruker Compass Profile Analysis 2.1 software for background noise subtraction, data reduction, and peak alignment to obtain a table of mass and retention time pairs with associated intensities for all detected peaks. The Profile Analysis parameters were set as follows, retention time range 0.1–18.9 min, mass range 100–1200 Da, and mass error window <25 ppm and retention time window 0.1 min. The marker intensities were normalized to the sum of the intensities in each sample to obtain the same total intensity of all the samples and data was processed by Pareto scaling and exported to Excel format and processed using MetaboAnalyst online software for data normalization and multivariate analysis.

4.1.8. Statistical analysis

All analyses, including physiochemical parameters (brix, pH, color), phytochemical contents (ascorbic acid, total ascorbic acid, nitrate, and total phenolics), and antioxidant activities (DPPH and ABTS assay), were conducted using JMP Pro12 statistical data analysis software (SAS, USA). The data were subjected to one-way analysis of variance (ANOVA) for mean comparison at $p < 0.05$. LC-MS data were subjected to multivariate statistical analysis including partial least square discriminant analysis (PLS-DA) and heatmaps were constructed using MetaboAnalyst 4.0 software (http://www.metaboanalyst.ca). All results were expressed as means ± SE.

4.2. Results and discussion

4.2.1. Variation in color attributes, brix, and pH

Colors in vegetables, fruits, and processed juices are important indicators of food quality which also reflect the levels of some health-promoting compounds, including antioxidants such as betalains, anthocyanins and carotenoids (Dominy, 2004). The values for color attributes (L, a*, b*, C, and h), pH, and °Brix of the 30 commercial juices are shown in Table A-3. In kale juices, the lightness* values ranged from 35.92 to 48.95. Grown Vegetable Juice (K3) and Healthy Greens (K2), had a* values of 9.64 and 0.61, respectively, indicating the presence of red color in these juices. The reddish color can be partly attributed to the lycopene and carotenoids in the tomato and carrot juices in Grown Vegetable Juice (K3). The overall chroma and hue values in cold-pressed juices were lower than those of thermally processed juices. It is possible that cold-pressed juices were less saturated than thermally processed juices, due to the larger quantities of solids retained in cold-pressed juice products.

In beet juices, L, a*, b*, C, and H* were variable based on the juice processing techniques and ingredients. For example, the V8 brand juices showed the highest brightness followed by cold-pressed juices such as Organic Beet (B9) and Cucum Berry (B10). Furthermore, dark purple colored pure beetroot juices such as Beet Juice (B5) and Pure Beet (B6) had the lowest L and a value, which reflected higher betalain contents.

Among all melon juices, Antioxidant Infusion Juice (M2) had a bright red color and was the only juice that had a negative b* value. Watermelon juices were generally high in lightness and redness values, which could be attributed to the presence of red colored lycopene. Among all Kiss Melon cold-pressed juices, Beet Boost Juice (M10) had the lowest L value and lower b* value due to presence of higher levels of betalain in beetroot juices.

Brix is another important factor affecting juice palatability. In general, among all juices, the sugar content varied from 3.20 (M2) to 14.47 (K1) °Brix (Table A-3). The pH of all juices ranged from 3.25 (M2) to 4.95 (M7), which gave the juices a bright, tangy taste.

4.2.2. Influence of juice ingredients and processing techniques on ascorbic acid, total ascorbic acid, and nitrate

The results of AA, total AA (TAA), and nitrate contents in 30 commercial juices with three categories were presented in table 1. Among kale juices (K1–K10), Healthy Greens (K2) had the highest AA (46.22 µg/mL), which was produced thermally by combining fruits and vegetable juices with vitamin C as a preservative and its price was the lowest compared to other kale juices in this study (Table A-2). Followed by thermally processed Green Machine (K1), which had 19.03 µg/mL AA that coming from plant source. In comparison, the cold pressed kale juices (K3-K10) depicted lower AA and showed no significant difference among them. According to nitrate content, cold pressed Radiant Probiotic Organic (K6) showed the highest levels (510.37µg/mL), followed by thermally obtained Healthy Greens (K2), with the value of 404.90 µg/mL. Considering that kale, celery and spinach are rich sources of nitrates, the results are in agreement with the ingredients found in the juices (Petersen & Stoltze, 1999).

Among the 11 various beetroot juices tested, V8 brand juices (B1-B4) had significantly higher amounts of AA and TAA than other beet juices, despite the prices were lower than its respective beet juices. The highest amounts of AA and TAA were found in Original 100% Vegetable Juice Low Sodium (B3) and Spicy Hot 100% Vegetable Juice (B4), with values of 660.59 and 656.76 µg/mL, respectively. Both juices contained vitamin C as a preservative, which contributed substantially to the amounts of AA and TAA. The highest amount (907.79 -1074.66

µg/mL) of nitrate was detected in pure beetroot juices, Pure Beet (B6) and Beet Juice (B5), suggesting that beetroot juice is a good source of dietary nitrate. Significant nitrate differences observed between the cold-pressed juices such as Organic Beet (B9) and Cucum Berry (B10), which is likely due to their different ingredients present in each juice.

Among melon juices, Antioxidant Infusion (M2) had the highest amount of AA (44.80 µg/mL). Honey Kiss juice (M8) showed the highest TAA content (276.80 µg/mL), which may be due to added vitamin C. The highest nitrate was detected in Melon Beet Boost juice (M9, 209.35 µg/mL), since the ingredients kale and spinach are rich in nitrate. The present research demonstrated that the fruit and vegetable juices are rich in AA, TAA, nitrate and their levels were influenced by the ingredients than the processing techniques. Ascorbic acid is one of the most important free radical scavengers, but it is found in both reduced form (ascorbic acid) and oxidized form (dehydroascorbic acid) After treating with reducing reagent TCEP, the dehydroascorbic acid has been converted to AA, the TAA was measured by HPLC. In addition, nitrate acts as a substrate for *in vivo* generation of nitric oxide in the human body. The consumption of nitrate provides NO-like effects, including reduction of blood pressure, inhibition of platelet aggregation, and vasoprotective activities (Lundberg, Carlström, Larsen, & Weitzberg, 2010).

4.2.3. Free radical scavenging activity and total phenolics

Antioxidant activity is generally measured by more than one assay based on the different principle. In the present study, DPPH and ABTS assays were used to test the ability of antioxidants in the juice samples to scavenge the free radicals. The ABTS cation radical is scavenged by hydrophilic and hydrophobic antioxidants, whereas DPPH is scavenged by the majority of hydrophobic compounds in presence of organic solvents (Floegel, Kim, Chung, Koo, & Chun,

2011). Therefore, these two assays test different aspects of the antioxidant activity of juice samples. The results of radical scavenging activity and total phenolics (TP) were presented in Appendix Fig.7. A significant variation in TP was observed among kale juices, with values ranging from 67.16 µg/mL to 172.05 µg/mL of gallic acid equivalents. Green Machine (K1), made up of 100% vegetables without additives or added water, showed the highest TP. The lowest TP was found in Farms Daily Greens (K1), which consisted of diluted mixed juices. In beetroot blended smoothies, Farms Daily Roots (B7), had a significantly higher content of TP (606.29 µg/mL) than other mixed juices. This result could be explained by Kujala and others (T. Kujala, Loponen, & Pihlaja, 2001) who compared the TP in different parts of beetroot and found the highest amount of TP was found in peel, followed by crown and flesh. In our study, the blended beet smoothies retained more byproducts include peel and pulps that had various phenolics, which significantly contributed to TP. In addition, the added purple carrot juice, which is rich in phenolic compounds, could be another reason that Farms Daily Roots (B7) juice had high TP. Furthermore, Beet Juice (B6) contained high TP contents due to the probiotic lactic acid bacteria (LAB) fermentation process, which converted complex phenolics into free forms and depolymerized high molecular weight phenolics by polyphenol oxidases. Additionally, acidification might have stabilized the phenolics, which led to the higher TP levels compared to other juices. The lowest TP was found in Very Veggie (105.56 µg/mL). In melon juices, the highest TP was found in Antioxidant Infusion Juice (M2) and the lowest TP was in Watermelon Juice (M3) due to the presence of only 10% of juice.

In this study, the antioxidant activities of 30 commercial juices were tested and among kale juices, Green Machine (K1) was the most potent (385.64 µg/mL) scavenger of the ABTS radical, followed by Healthy Greens (K2, 243.67 µg/mL). The other mixed juices had a moderate

inhibitory effect, ranging from 63.91 µg to 180.08 µg of AA equivalents per mL of juice. Beetroot juices depicted efficient scavenging of the ABTS radical ranging from 164.00 µg/mL to 524.00 µg/mL, and the highest value was found in Farms Daily Roots (B7). The juice with the lowest value was Organic Beet (B9), which was obtained by blending beet, carrot, orange, and lemon juice, had an ABTS value of 164.00 µg/mL. In melon juices, the ABTS radical ranging from 24.52 µg/mL to 456.28 µg/mL. Antioxidant Infusion (M2) was the most effective scavenger of the ABTS radical and Watermelon Juice (M3) had the lowest activity among all tested juices. Similar to the ABTS results, in kale juices, the highest DPPH value was obtained in Green Machine (K2) with AA equivalents of 265.69 µg/mL of juice. Among melon juices, the levels of DPPH ranged from 75.82 to 317.91 µg/ mL AA equivalents, with the lowest DPPH activity found in Watermelon Juice (M3), and the highest was found in Antioxidant Infusion (M2). The highest DPPH radical scavenging activity was observed in pure beetroot juices such as Beet Juice (B5) and Pure Beet (B6), with DPPH values of 619.08 µg/mL and 544.67 µg/mL of AA equivalents, respectively. Similar results were reported by Wootton-Beard, Moran, and Ryan,(Wootton-Beard & Ryan, 2011) who investigated antioxidant activities of 23 commercial juices with different types and ingredients. The authors demonstrated that beetroot juice had the highest DPPH and ABTS radical scavenging activities compared to other pure or mixed commercial juices.

In the present study, the discrepancies between the DPPH and ABTS radical scavenging activities were observed. It is possible that the nature of antioxidants present in each juice determines levels of ABTS and DPPH. Furthermore, different DPPH and ABTS results help to determine the discrepancies of reaction kinetic mechanisms between these assays (G. K. Jayaprakasha, Girennavar, & Patil, 2008). According to previous study (Schaich, Tian, & Xie, 2015) two types of radical scavenging mechanisms, hydrogen atom transfer (HAT) and single

electron transfer (SET), occur in the food matrix. The ABTS radical quenching reaction was generally initiated by SET, which is a fast and efficient reaction system, whereby antioxidants donate two electrons that allow full access for ABTS radical site within milliseconds. Afterwards, the reaction is impeded as the adduction of phenolic rings and acid groups hinders the active site. In contrast, DPPH reactions are more complicated and mostly attributed to the slow HAT mechanism, unless some potent hydrogen-bonding solvents interfere with the release of hydrogen atoms, which promotes SET over HAT in certain samples. Furthermore, certain compounds (AA and phenolics) autoxidize oxygen to $O_2^{\cdot-}$, which reacts rapidly with ABTS radicals. In the DPPH solvent system, low antioxidant activity may result from weak reactions.

4.2.4. Pearson correlation analysis for variables

Pearson correlation analysis was performed to determine relationship among variables. As shown in Table 4, the Pearson correlation coefficients (r) of TP with DPPH and ABTS assays in kale juices were positive and high (r = 0.7993 with DPPH, r = 0.7842 with ABTS), suggesting that phenolic compounds were the main components responsible for free radical scavenging activities. AA exhibited a moderate positive correlation with TAA (r=0.7532), DPPH (r=0.3117), and ABTS (r=0.5309). These results suggested that phenolic compounds and AA contributed to the free radical scavenging activities of kale juices. Kale juices had numerous phenolic compounds and their higher antioxidant activities were possibly due to the number of acidic, phenolic hydroxyl groups, aromatic rings and free electron pairs on the phenolic oxygen, which induced an increase in electron delocalization (Miller & Rice-Evans, 1997). In beetroot juices, the correlations between TP with DPPH (r = 0.9025) and ABTS results were also high (r = 0.7803). However, the AA and TAA were low or negatively correlated to those assays (r = -0.1503 and 0.1102). These results

suggested that phenolic compounds were main contributors to DPPH and ABTS free radical scavenging activities in beetroot juices, which agreed to previous research (Bavec, Turinek, Grobelnik-Mlakar, Slatnar, & Bavec, 2010). In melon juices, high and positive correlations were found for TP with DPPH (r = 0.7239) and ABTS (r = 0.7625) assays, but moderate correlations were observed between AA and TAA with these assays, suggesting phenolic compounds were main contributors in melon juices. Ascorbic acid has been considered as effective free radical scavengers, whereas the contribution to total antioxidant activities varied in different juice products, with percentage ranging from <5% in apple, pineapple, and vegetable juices, to 65–100% of citrus juice (Gardner, White, McPhail, & Duthie, 2000). Among all the assays, the correlation between DPPH and ABTS assay was higher than 0.9, suggesting both assays were suitable for detecting free radical scavenging activities in all 30 juices, despite the discrepancies of reaction mechanism. Our results suggest that it is difficult to specify the component responsible for antioxidant activities since the ratio of ingredients were not provided on the juice samples.

4.2.5. Untargeted metabolomics analysis in commercial juice samples

In the present study, UPLC-HR-ESI-QTOF-MS analysis was performed to obtain retention times, accurate molecular masses, and MS/MS fragmentations for the characterization of metabolites to discriminate the juice samples. Table 5 summarizes the molecular ion adducts, accurate mass and major MS/MS fragments of identified compounds in the juices. In the present study, we identified 48 metabolites representing three classes, i.e., flavonoids, phenolic acids, and betalains, in kale, beet, and melon juices. Kale juices mainly included flavonoid derivatives of kaempferol and quercetin. For instance, we tentatively identified quercetin-3-diglucoside-7-glucoside (m/z 788), kaempferol-3-O-diglcoside-7-glucoside (m/z 772), kaempferol-3-(feruoyl)-

sophoroside (m/z 934), quercetin-3-feruoyl-diglucoside-7-diglucoside (m/z 1126), kaempferol-3-feruloyl-diglucoside-7-diglucoside (m/z 948), and kaempferol-3-feruloyl-diglucoside-7-diglucoside (m/z 1110). The major health-promoting compounds found in beet juices include betacyanin derivatives in which betanidin (m/z 177) was the aglycone for most of the juices (Azeredo, 2009). A total of 23 compounds were identified in different 11 commercial beet juices. In addition to 14 betalains, 5 phenolics were found, which were 1,3-Dicaffeoylquinic acid (m/z 517), chlorogenic acid (isomer) (m/z 355), caffeoylquinic acid (m/z 731), ferulic acid derivative (m/z 519). Further, melon juices predominantly contained quercetin (m/z 303) and kaempferol (m/z 287) derivatives due to the blending of different juices including grape extract. A total of 11 compounds were identified in various melon juices tested in the present study. Interestingly, phenylalanine was found in all three juice categories.

4.2.6. Influence of processing techniques on phenolic compounds

In recent years, chemometrics has emerged as an efficient tool used in food industries to classify products, evaluate food quality, and detect food authenticity. In this section, chemometric methods OPLS-DA (Fig. 4A-C) was performed and perfectly separated thermal and cold processed kale, beet, and juices, and heatmap provides an overview of abundance of each metabolite.

According to the heatmap of kale juices (Fig 4A), most of the cold-pressed kale juices were abundant in kaempferol and quercetin glycosides. For instance, cold-pressed Organic Emerald Green (K8) was abundant in quercetin-3-diglucoside-7-glucoside and its isomer (m/z 789), kaempferol-3-sinapoyl-diglucoside-7-glucoside (m/z 979), kaempferol-3-feruloyl-diglucoside7-diglucoside (m/z 1111) and epigallocatechin (m/z 307). This juice also contained spinacetin 3-O-glucosyl-(1-6)-glucoside (m/z 671) attributed to the presence of spinach as one of the ingredients

(Barkat, Singh, Jayaprakasha, & Patil, 2018). Similarly, cold-pressed Grown Vegetable Juice (K3) made of kale, spinach, yellow carrot, tomato and other fruits or vegetables, showed the presence of kaempferol-3-O-caffeoyl-sophoroside (m/z 935), kaempferol-3-O-sophoroside (m/z 611), gallocatechin (m/z 307), and phenylalanine (m/z 166). However, thermal-processed Healthy Greens (K2) had medium levels of quercetin-3-diglucoside-7-glucoside isomer (m/z 789), sinapoyl-diglucoside-7-glucoside (m/z 979), and kaempferol-3-sinapoyl-diglucoside-7-glucoside (m/z 979), indicating those compounds could be considered as potential metabolites screening the difference of thermal and cold processed kale juices.

The heatmap of beet juices discriminated the cold-pressed and thermally processed juices by identifying certain compounds such as 2, 17-bidecarboxy-2,3-dehydro-neobetanin and its isomer (m/z 459), 2-decarboxy-2,3-dehydro-neobetanin (m/z 503), and isobetanin (m/z 551). These compounds were significantly higher in thermal-processed beet juices compared with cold-pressed juices (Fig. 4B). In cold-pressed beet juices, Organic Beet (B9) and Organic Cucum Berry (B10), we found only two phenolic compounds, namely caffeolyquinic acid (m/z 731) and a ferulic acid derivative (m/z 503), in relatively higher amounts compared to other compounds. In sum, the betalains (betacyanins and betaxanthins) and derivatives, which were abundant in thermally processed juices, could be considered as potential health-promoting compounds based on their high abundance and unique existence in cold-pressed juices.

In the melon juice heatmap (Fig 4C), thermal processed Synergy Watermelon Wonder (M1) was distinguished from other melon juices by its various ingredients such as watermelon, cherry, lime, and tea extract. This juice was abundant in kaempferol and quercetin glycosides, namely quercetin 3-rhamnosyl (1→2)-rhamnosyl-glucoside (m/z 757), kaempferol 3-O-(2′′-rhamnodyl-galatoside)-7-O-rhamnoside (m/z 741), kaempferol 3-O-glucosyl-rhamnosyl-

galatoside (m/z 757), kaempferol 3-O-glucosyl-rhamnosyl-glucoside (m/z 757). Another thermally processed juice, antioxidant infusion (M2), had significantly higher epigallocatechin 3-O-gallate (m/z 459). In cold-pressed melon juices, phenylalanine (m/z 166) was found in each juice, except for Honey Kiss Juice (M7), which was rich in quercetin 3-O-galactoside 7-O-rhamnoside (m/z 611) and quercetin 3-O-rhamnosyl-galactoside (m/z 611). Summer Kiss Juice (M6), a cold-pressed juice, also had those compounds along with kaempferol 3-O-galactoside-7-O-rhamnoside (m/z 595).

To investigate the differences of other parameters such as TP and antioxidant activities of juices with thermal and cold process techniques, partial least square discriminant analysis (PLS-DA) coupled to whisker plots was conducted. PLS-DA, an effective pattern recognition technique used to discriminate samples, was used to determine the significance of group separations, and search for potential health-promoting compounds. In each juice type, PLS-DA separated most cold-pressed and thermally processed juices (Fig.5A–C), except for a few samples were clustered or close, which had similar physiochemical attributes, phytochemical contents, or free radical scavenging activities. Thermal-processed kale, beet, and melon juices had higher total phenolics, DPPH, and ABTS values. Table 5 shows the identified kaempferol and quercetin conjugated glucosides in kale and melon juices. During thermal processing, the sugar moiety in flavonoid glycosides, might be cleaved in kale and melon juices providing more free hydroxyl groups, which will increase the phenolics and radical scavenging activities. Therefore, thermal-processed kale and melon juices had higher phenolics and radical scavenging activities compared to cold-pressed juices. Kaempferol and quercetin derivatives are powerful antioxidants due to their hydroxy groups and the unsaturation in the C ring, which allowed electron delocalization across the molecules (Rice-Evans, Miller, & Paganga, 1996). Another study investigated the effect of thermal

processing methods on antioxidant activities of red beet, and they found an increase after the treatment, which agreed with the results found in our study (Ravichandran, Saw, Mohdaly, Gabr, Kastell, Riedel, et al., 2013).

4.2.7. Influence of production system on phenolic compounds

In recent years, consumers have increasingly preferred organic food. Although numerous studies have been conducted to determine the levels of antioxidant activities of organic and conventional crops, the results were not conclusive (Kazimierczak, Hallmann, Lipowski, Drela, Kowalik, Püssa, Matt, Luik, Gozdowski, & Rembiałkowska, 2014; Uckoo, Jayaprakasha, & Patil, 2015). In the present study, PLS-DA was applied to classify the juices obtained from conventional and organic production systems based on their TP and antioxidant activities. The PLS-DA 3D plot (Appendix Fig S8 A–C) showed two distinct clusters for conventional and organic production systems in kale, beet, and melon juices. Box and whisker plots demonstrated that commercial beet juices obtained from organically grown crops had higher TP, DPPH, and ABTS levels, whereas kale and melon juices acquired from conventionally grown crops had higher levels of phenolics, DPPH, and ABTS. Similar findings reported by Bavec and others (Bavec, Turinek, Grobelnik-Mlakar, Slatnar, & Bavec, 2010) showed that TP and DPPH activity were significantly higher in organically grown beets than in conventionally beets. Kazimierczak and coauthors found organic beetroots contained significantly higher amounts of specific phenolic compounds and antioxidant activities (Kazimierczak, Hallmann, Lipowski, Drela, Kowalik, Püssa, Matt, Luik, Gozdowski, Rembiałkowska, et al., 2014). Additionally, Unal, Susanti, and Taher found significantly higher polyphenol content and antioxidant activities in conventionally grown *Brassica* leafy vegetables compared to the organically grown extracts (Unal, Susanti, & Taher, 2014). The authors explained

that the discrepancies in these results could be due to environmental factors such as season, geography, or agronomic practices.

4.3. Conclusion

The present study reports that the processing techniques, production system, and ingredients will affect significantly the phytochemical composition and antioxidant activities. Untargeted UPLC-HR-ESI-QTOF-MS based metabolomics data revealed the presence of 48 metabolites including 27 flavonoids, 14 betalain derivatives, 5 phenolics and 2 free amino acids in 30 commercial juices. Multivariate statistical analysis illustrated that kaempferol and quercetin glycosides, decarboxylated betalains, and quercetin derivatives were could be used as potential markers in discriminating the processing techniques, production system, and ingredients in those kale, beet, and melon juices, separately.

Furthermore, results of antioxidant activities and phytochemical contents highlighted the health benefits of all the juices whereas the results were more affected by ingredients than processing techniques, suggesting the importance of analyzing food components. This study demonstrated that a metabolomic analysis, coupled to chemometric tools has potential as a fast and efficient approach for discriminating the processing techniques of commercial juices.

Table 3. Results* for ascorbic acid, total ascorbic acid, and nitrate content in 30 commercial juices.

Type**	Code	Juice and Brand Names	Ascorbic acid	Total ascorbic acid	Nitrate
Kale Juices	K1	Green Machine (Naked)	19.03 ± 0.71^{b}	89.24 ± 1.00^{a}	13.91 ± 0.51^{h}
	K2	Healthy Greens (V8)	46.22 ± 2.51^{a}	67.86 ± 2.53^{b}	404.90 ± 4.51^{b}
	K3	Grown Vegetable Juice (Estate)	3.45 ± 0.05^{c}	6.85 ± 0.08^{g}	318.03 ± 1.64^{c}
	K4	Green Delight Organic (Suja)	5.06 ± 0.20^{c}	8.13 ± 0.06^{fg}	49.34 ± 1.76^{g}
	K5	Uber Greens Organic (Suja)	3.32 ± 0.04^{c}	22.17 ± 0.79^{c}	208.45 ± 0.53^{e}
	K6	Radiant Probiotic Organic (Suja)	3.79 ± 0.08^{c}	18.08 ± 0.55^{cd}	510.37 ± 2.22^{a}
	K7	Organic Kale (1915)	4.05 ± 0.13^{c}	9.23 ± 0.11^{fg}	230.03 ± 4.30^{d}
	K8	Organic Emerald greens (Evolution)	3.08 ± 0.02^{c}	15.59 ± 0.35^{de}	164.25 ± 0.14^{f}
	K9	Organic Green Devotion (Evolution)	4.68 ± 0.06^{c}	11.56 ± 0.28^{ef}	397.03 ± 1.79^{b}
	K10	Super Fruit Greens (Evolution)	6.57 ± 0.17^{c}	10.24 ± 0.13^{fg}	20.48 ± 0.31^{h}
Beet Juices	B1	Strawberry Banana (V8)	243.05 ± 10.38^{b}	427.80 ± 28.59^{d}	93.65 ± 0.29^{b}
	B2	Original 100% Vegetables juice (V8)	219.93 ± 25.54^{b}	1003.25 ± 50.66^{b}	79.80 ± 4.25^{c}
	B3	Original 100% Vegetables Juice Low Sodium (V8)	660.59 ± 8.82^{a}	803.83 ± 15.74^{c}	85.13 ± 10.00^{c}
	B4	Spicy Hot 100% Vegetables Juice (V8)	656.71 ± 2.69^{a}	1182.86 ± 96.13^{a}	86.71 ± 9.90^{c}
	B5	Beet Juice (Biotta)	2.59 ± 0.05^{c}	4.86 ± 0.03^{e}	907.79 ± 8.37^{a}
	B6	Organic Pure Beet (Lakewood)	3.93 ± 0.08^{c}	5.30 ± 0.27^{e}	1074.66 ± 8.48^{a}
	B7	Farms Daily Roots (Bolthouse)	3.38 ± 0.04^{c}	261.8 ± 28.3^{d}	512.12 ± 3.42^{c}
	B8	Very Veggie (R.W. Knudsen)	3.16 ± 0.08^{c}	29.78 ± 1.03^{e}	18.63 ± 1.56^{c}
	B9	Organic Beet (1915)	4.78 ± 0.08^{c}	13.60 ± 0.21^{e}	359.35 ± 5.05^{b}
	B10	Organic Cucum Berry (Simple Truth)	3.80 ± 0.07^{c}	26.55 ± 0.87^{e}	41.96 ± 0.59^{c}
Melon Juices	M1	Synergy Watermelon Wonder (GTS)	2.61 ± 0.04^{e}	12.03 ± 0.39^{f}	3.07 ± 0.37^{g}
	M2	Antioxidant Infusion (Bai)	44.80 ± 0.56^{a}	50.44 ± 0.77^{e}	5.82 ± 0.64^{fg}
	M3	Watermelon (Tropicana)	2.72 ± 0.01^{e}	6.62 ± 0.03^{f}	5.79 ± 0.47^{fg}
	M4	MLN Watermelon (WTR)	3.35 ± 0.03^{de}	11.15 ± 0.11^{f}	60.12 ± 0.64^{c}
	M5	Sugar Kiss Juice Raspberry (Kiss Melon)	7.79 ± 0.66^{b}	159.15 ± 1.66^{c}	42.15 ± 0.33^{d}
	M6	Summer Kiss Juice (Kiss Melon)	5.22 ± 0.04^{c}	244.09 ± 6.14^{b}	49.16 ± 2.28^{cd}
	M7	Honey Kiss Juice (Kiss Melon)	4.30 ± 0.18^{cd}	276.80 ± 13.65^{a}	18.65 ± 0.18^{ef}
	M8	Sugar Kiss Juice Apple (Kiss Melon)	3.51 ± 0.07^{de}	18.56 ± 0.26^{f}	27.40 ± 0.49^{e}
	M9	Melon Garden Greens Juice (Kiss Melon)	3.30 ± 0.03^{de}	160.52 ± 1.59^{c}	209.35 ± 9.22^{a}
	M10	Melon Beet Boost Juice (Kiss Melon)	3.06 ± 0.03^{de}	130.81 ± 1.00^{d}	108.31 ± 0.27^{b}

*Values (μg/mL) are expressed as mean ± SE from two biological experiments with three replicates

** The above juices were categorized based on assumption that those juices may contain the major amount of respective juices (kale, beet, melon). However, labels do not state the composition of each juice.

Table 4. Pearson's correlation coefficients (r) of color parameters, phytochemical contents, and antioxidant activities in commercial kale, beet, and melon juices.

		DPPH	TP	ABTS	AA	TAA	Nitrate
Kale Juices	DPPH	1.0000					
	TP	0.7994	1.0000				
	ABTS	0.9351	0.7842	1.0000			
	AA	0.4943	0.3117	0.5309	1.0000		
	TAA	0.7721	0.4767	0.7495	0.7532	1.0000	
	Nitrate	-0.4330	-0.3962	-0.3374	0.1425	-0.1476	1.0000
Beet Juices	DPPH	1.0000					
	TP	0.9025	1.0000				
	ABTS	0.9043	0.7803	1.0000			
	AA	-0.1503	-0.4059	0.1102	1.0000		
	TAA	-0.0849	-0.3143	0.2278	0.8409	1.0000	
	Nitrate	0.6798	0.6916	0.4246	-0.4612	-0.5175	1.0000
Melon Juices	DPPH	1.0000					
	TP	0.7239	1.0000				
	ABTS	0.9203	0.7625	1.0000			
	AA	0.6015	0.5802	0.4989	1.0000		
	TAA	0.3714	0.5459	0.3619	0.8615	1.0000	
	Nitrate	-0.3681	-0.5036	-0.4100	-0.2980	-0.1414	1.0000

Abbreviations: TP: total phenolics; AA: ascorbic acid; TAA: total ascorbic acid.

Table 5. Characteristic marker compounds identified by UPLC-HR-MS in various juice samples.

RT (min)	Identified compound	Molecular formula	Experimental mass	Adduct	Theoretical mass	Mass error (ppm)	MS/MS fragments	Juice Type			Reference
								Kale	Beet	Melon	
1.40	Epigallocatechin	$C_{15}H_{14}O_7$	307.0925	$[M + H]^+$	307.0812	36.80	230, 84	X			(Bresciani, Calani, Cossu, Mena, Sayegh, Ray, et al., 2015)
2.65	2,17-Bidecarboxy-2,3-dehydro-neobetanin	$C_{22}H_{22}N_2O_9$	459.1601	$[M + H]^+$	459.1398	44.2	330, 284, 203		X		(Nemzer, et al., 2011)
3.50	Phenylalanine	$C_9H_{11}NO_2$	166.0850	$[M + H]^+$	166.0863	-7.83	103	X	X	X	(Ma, Tian, Luo, Zhou, Sun, & Ma, 2013)
4.20	1,3-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	517.1558	$[M + H]^+$	517.1341	42.0	452, 325, 136		X		
5.75	L-Tryptophan	$C_{11}H_{12}N_2O_2$	205.0960	$[M + H]^+$	205.0972	-5.9	-		X		
6.00	Chlorogenic acid	$C_{16}H_{18}O_9$	355.1028	$[M + H]^+$	355.1024	1.1	163		X		(Georgiev, Weber, Kneschke, Denev, Bley, & Pavlov, 2010)
6.05	Chlorogenic acid isomer	$C_{16}H_{18}O_9$	355.1025	$[M + H]^+$	355.1024	0.3	163		X		
6.20	Betanin	$C_{24}H_{26}N_2O_{13}$	551.1592	$[M + H]^+$	551.1508	15.2	389, 150		X		(Nemzer, et al., 2011)
6.30	2'-O-Glucosyl-betanin/isobetanin	$C_{30}H_{37}N_2O_{18}^+$	713.2042	$[M + H]^+$	713.2036	0.8	551, 389		X		
6.45	Isobetanin	$C_{24}H_{26}N_2O_{13}$	551.1559	$[M + H]^+$	551.1508	9.3	389, 298, 136		X		
6.55	17-Decarboxy-isobetanin	$C_{23}H_{26}N_2O_{11}$	507.1659	$[M + H]^+$	507.1609	9.9	345		X		
6.65	15-Decarboxy-betanin	$C_{23}H_{26}N_2O_{11}$	507.1652	$[M + H]^+$	507.1609	8.5	345		X		
7.20	2-Decarboxy-isobetanin	$C_{23}H_{26}N_2O_{11}$	507.1655	$[M + H]^+$	507.1609	9.1	345		X		
7.30	2,17-Bidecarboxy-betanidin/isobetanidin	$C_{22}H_{27}N_2O_9^+$	463.1752	$[M + H]^+$	463.1711	8.9	301		X		
7.60	2-Decarboxy-betanin	$C_{23}H_{26}N_2O_{11}$	507.1649	$[M + H]^+$	507.1609	7.9	345		X		
7.60	Caffeoylquinic acid	$C_{16}H_{18}O_9$	731.1883	$[M + H]^+$	731.1794	12.2	377, 163		X		(Yang, Jayaprakasha, & Patil, 2018b)
7.65	Quercetin-3-diglucoside-7-glucoside	$C_{33}H_{40}O_{22}$	789.1884	$[M + H]^+$	789.2084	-25.3	541, 435, 387, 303	X			

Table 5. Continued

RT (min)	Identified compound	Molecular formula	Experimental mass	Adduct	Theoretical mass	Mass error (ppm)	MS/MS fragments	Juice Type			Reference
								Kale	Beet	Melon	
7.70	Neobetanin	$C_{24}H_{24}N_2O_{13}$	549.1414	$[M + H]^+$	549.1351	11.5	287, 341		X		(Nemzer, et al., 2011)
7.85	17-Decarboxy-neobetanin	$C_{23}H_{24}N_2O_{11}$	505.1515	$[M + H]^+$	505.1453	12.3	343, 297		X		
8.00	δ-Viniferin	$C_{28}H_{22}O_6$	455.1410	$[M + H]^+$	455.1489	-17.4	367, 195		X		(Vitaglione, Sforza, & Del Rio, 2012)
8.15	Kaempferol-3-O-diglucoside-7-glucoside	$C_{33}H_{40}O_{21}$	773.1958	$[M + H]^+$	773.2135	-22.9	633, 395, 287	X			(Yang, Jayaprakasha, & Patil, 2018b)
8.55	Kaempferol-3-O-(caffeoyl)-sophoroside	$C_{42}H_{46}O_{24}$	935.2477	$[M + H]^+$	935.2452	2.7	737, 633, 463, 287	X			(Lin & Harnly, 2009)
8.90	2-Decarbxy-neobetanin	$C_{23}H_{24}N_2O_{11}$	505.1521	$[M + H]^+$	505.1453	13.5	343, 297		X		(Nemzer, et al., 2011)
9.05	2,17-Bidecarboxy-2,3-dehyo-neobetanin (isomer)	$C_{22}H_{22}N_2O_9$	459.1414	$[M + H]^+$	459.1398	3.5	297		X		
9.10	Epigallocatechin 3-O-gallate	$C_{22}H_{18}O_{11}$	459.0906	$[M + H]^+$	459.0922	-3.5	287, 139			X	(Davis, Cai, Davies, & Lewis, 1996)
9.40	Quercetin-3-feruloyl-diglucoside-7-diglucoside	$C_{49}H_{58}O_{30}$	1127.2766	$[M + H]^+$	1127.3086	-28.4	987, 825, 523, 406, 303	X			(Yang, Jayaprakasha, & Patil, 2018b)
9.80	Kaempferol-3-feruloyl-digulcoside-7-diglucoside	$C_{49}H_{58}O_{29}$	1111.2821	$[M + H]^+$	1111.3137	-28.4	809, 541, 411, 287, 177	X			
9.85	Kaempferol-3-(feruoyl)-sophoroside-7-O-glucoside	$C_{43}H_{48}O_{24}$	949.2327	$[M + H]^+$	949.2608	-29.6	809, 707, 523, 411, 339, 287, 177	X			
9.85	Ferulic acid derivative		519.2527						X		(Kammerer, Carle, & Schieber, 2004)
10.10	2-Decarboxy-2,3-dehydro-neobetanin	$C_{23}H_{23}N_2O_{11}^+$	503.1317	$[M + H]^+$	503.1296	4.2	341		X		(Nemzer, et al., 2011)
10.95	Quercetin-3-diglucoside-7-glucoside (isomer)	$C_{33}H_{40}O_{22}$	789.2026	$[M + H]^+$	789.2084	-7.3	651, 541, 407, 303	X			(Yang, Jayaprakasha, & Patil, 2018b)
11.45	Kaempferol-3-O-sophoroside	$C_{27}H_{30}O_{16}$	611.1458	$[M + H]^+$	611.1607	-24.4	287	X			
11.60	Kaempferol-3-O-diglucoside-7- glucoside (Isomer)	$C_{33}H_{40}O_{21}$	773.1970	$[M + H]^+$	773.2135	11.6	633, 425, 287	X			
11.70	Quercetin 3-O-galactoside 7-O-rhamnoside	$C_{27}H_{30}O_{16}$	611.1593	$[M + H]^+$	611.1607	-2.3	413, 303, 149			X	

Table 5. Continued

RT (min)	Identified compound	Molecular formula	Experimental mass	Adduct	Theoretical mass	Mass error (ppm)	MS/MS fragments	Juice Type			Reference
								Kale	Beet	Melon	
11.85	Kaempferol-3-sinapoyl-diglucoside-7-diglucoside	$C_{50}H_{60}O_{30}$	1141.2915	$[M + H]^+$	1141.3242	-28.7	879, 619, 287, 129	X			(Yang, Jayaprakasha, & Patil, 2018b)
11.85	Eriocitrin/Neoeriocitrin	$C_{27}H_{32}O_{15}$	597.1712	$[M + H]^+$	597.1814	-17.08	463, 365, 289, 153	X			
11.90	Quercetin 3-O-rhamnosyl-galactoside	$C_{27}H_{30}O_{16}$	611.1587	$[M + H]^+$	611.1607	-3.3	413, 303, 149			X	
12.00	Quercetin 3-O-rutinoside	$C_{27}H_{30}O_{16}$	611.1458	$[M + H]^+$	611.1607	-24.4	555, 303		X		
12.10	Quercetin 3-rhamnosyl (1→2)-rhamnosyl-glucoside	$C_{33}H_{40}O_{20}$	757. 2156	$[M + H]^+$	757.2186	-4.0	633, 487, 303, 240, 185			X	(McDowell, Bailey, & Howard, 1990)
12.45	Isorhamnetin 3-O-diglucoside	$C_{28}H_{32}O_{17}$	641.1742	$[M + H]^+$	641.1712	4.68	317, 84	X			(Lin & Harnly, 2009)
12.50	Kaempferol 3-O-glucosyl-rhamnosyl-galactoside	$C_{33}H_{40}O_{20}$	757.2182	$[M + H]^+$	757.2186	-0.53	525, 413, 287, 149			X	(McDowell, Bailey, & Howard, 1990)
12.60	Kaempferol 3-O-glucosyl-rhamnosyl-glucoside	$C_{33}H_{40}O_{20}$	757.2167	$[M + H]^+$	757.2186	-2.5	525, 413, 287, 149			X	(Kelebek, 2016)
12.70	Kaempferol 3-O-(2’’-rhamnosyl-galatoside)-7-O-rhmnoside	$C_{33}H_{40}O_{19}$	741.2182	$[M + H]^+$	741.2237	-7.4	617, 525, 413, 287, 203			X	
12.95	Spinacetin 3-O-glucosyl-(1-6)-glucoside	$C_{29}H_{34}O_{18}$	671.1846	$[M + H]^+$	671.1818	4.17	411, 347, 269, 140	X			(Barkat, Singh, Jayaprakasha, & Patil, 2018)
13.15	Kaempferol 3-O-galactoside-7-O-rhamnoside	$C_{27}H_{30}O_{15}$	595.1626	$[M + H]^+$	595.1657	-5.2	487, 331, 287			X	(Yang, Jayaprakasha, & Patil, 2018b)
13.60	Naringin	$C_{27}H_{32}O_{14}$	581.1886	$[M + H]^+$	581.1865	3.61	271, 153	X			(Sethiya, Trivedi, & Mishra, 2014)
13.90	Kaempferol-3-sinapoyl-diglucoside-7-glucoside	$C_{44}H_{50}O_{25}$	979.2417	$[M + H]^+$	979.2714	-30.3	855, 655, 523, 347, 287	X			(Yang, Jayaprakasha, & Patil, 2018b)
14.15	Qucertin-3-O-rutinoside	$C_{27}H_{30}O_{16}$	611.1822	$[M + H]^+$	611.1607	46.30	265, 303, 153	X			(Yang, Jayaprakasha, & Patil, 2018b)

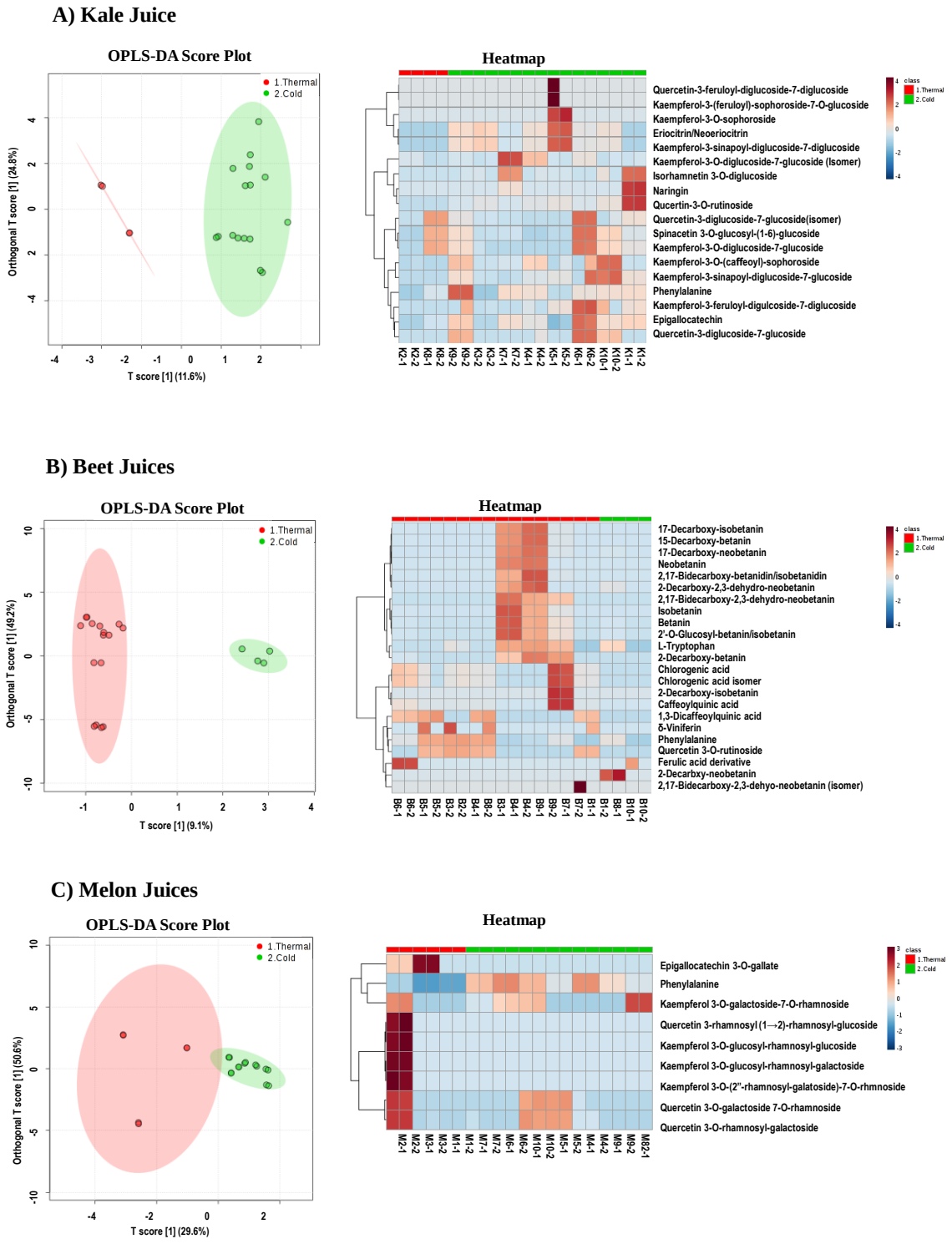

Figure 4. OPLS-DA score plot and heatmap based on LC-MS profiling of juice samples obtained from thermal and cold processed techniques. Color saturation in heatmap represents level of each metabolite with blue: the lowest; red: the highest.

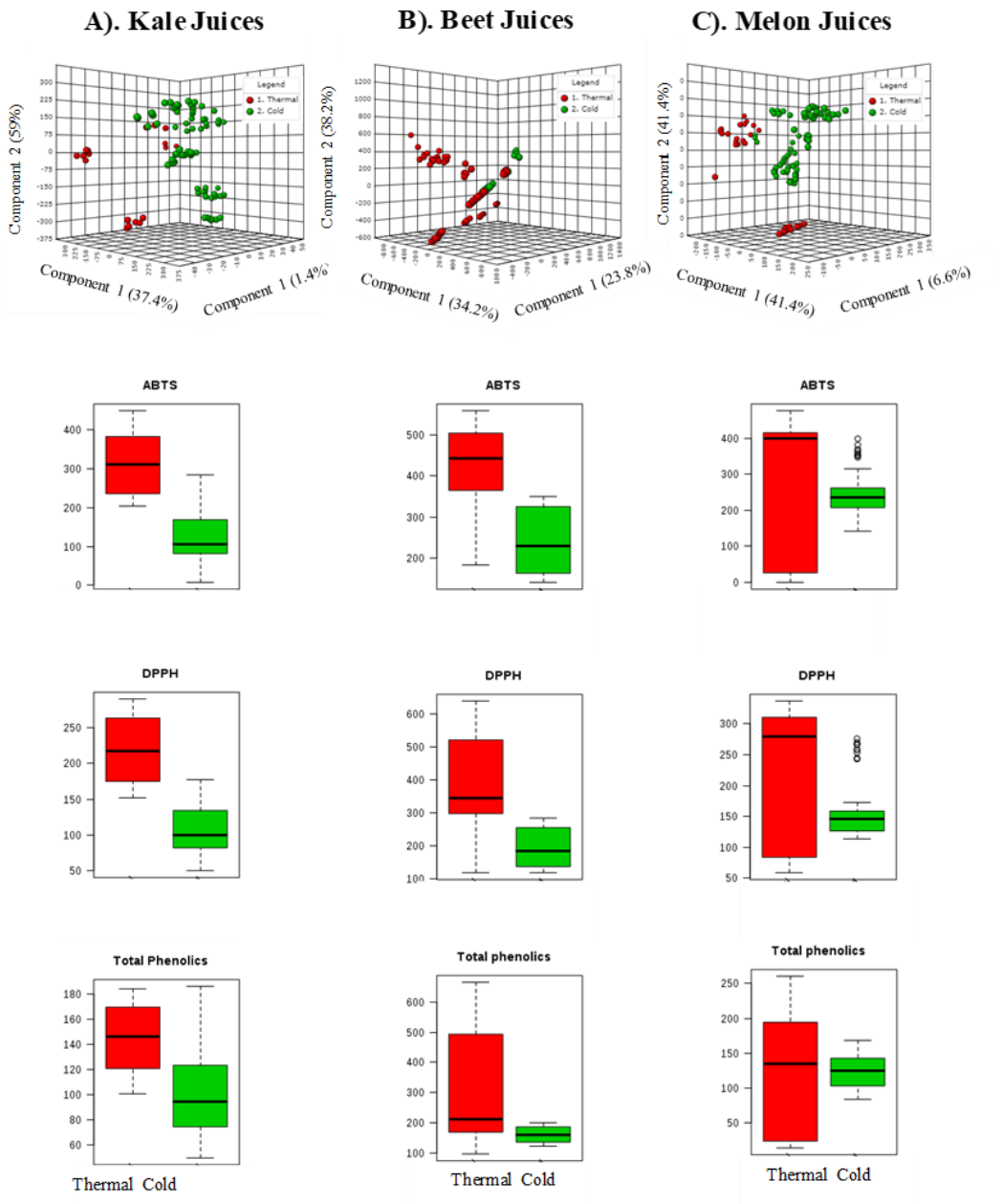

Figure 5. PLS-DA-3D score plot shows the antioxidant activities and total phenolics present in 30 commercial juice samples from (A) kale, (B) beet and (C) melon. Results were expressed as ascorbic acid (AA) equivalents, and total phenolics were expressed as gallic acid (GA) equivalents. Results of DPPH and ABTS were expressed as gallic acid (GA) equivalents. The red clustered dots were thermal processed juices, and the green grouped dots were cold processed juices.

5. UPLC-QTOF-MS FINGERPRINTING COMBINED WITH CHEMOMETRICS TO ASSESS THE SOLVENT EXTRACTION EFFICIENCY AND ANTIOXIDANT ACTIVITIES OF BEETROOT (*BETA VULGARIS*)

Epidemiological studies have demonstrated that the excessive production of reactive oxygen species (ROS) induces oxidative stress and increases the risk of chronic diseases such as cancer, diabetes, and cardiovascular diseases (Alfadda & Sallam, 2012; Liou & Storz, 2010). These studies also indicate that fruits and vegetables rich in bioactive compounds play a crucial role in the prevention of some chronic diseases and may provide desirable alternatives to some aspects of synthetic medicine due to their better compatibility with and fewer side effects to the human body (Sen, Chakraborty, Sridhar, Reddy, & De, 2010).

Beetroots (*Beta vulgaris*) are common vegetables cultivated worldwide and the increasing interest of consumers in the nutritional and heath-beneficial aspects of food has increased interest in this functional food (Wruss, Waldenberger, Huemer, Uygun, Lanzerstorfer, Müller, et al., 2015). Beetroots have significant antioxidant activities due to the presence of betalain derivatives and phenolic compounds. These compounds remove free radicals and protect the human body against damage from ROS (Sawicki, Bączek, & Wiczkowski, 2016). Betalains are effective antioxidants due to the presence of phenolic hydroxyl groups, aromaticity, and other characteristic functional groups (Gandía-Herrero, Escribano, & García-Carmona, 2009). Betalains consist of red-violet betacyanins and yellow-orange betaxanthins, which are biosynthesized from tyrosine (Sawicki, Bączek, & Wiczkowski, 2016). In addition to betalains, beetroots are rich in nitrate and other phytochemicals and the composition and levels of these compounds vary in different varieties of beets according to their phenotypes and genotypes. For example, red beetroot mainly contains betacyanins, whereas golden beetroot mainly contains betaxanthin.

Betalain levels, composition, and antioxidant activities are influenced by genetics, cultivars, production system, and processing techniques (Ravichandran, et al., 2013; Slatnar, Stampar, Veberic, & Jakopic, 2015b). Recently, increasing consumption of organically grown foods has been associated with the consumer interest in healthier and safer foods. Compared to conventionally grown foods, the organically grown food production system uses natural manures instead of artificial fertilizers, potentially resulting in more ingestion of health-promoting phytochemicals, allowing organic food to be perceived as healthier and safer than conventional foods (Krejčová, Návesník, Jičínská, & Černohorský, 2016). Previous studies demonstrated beetroots grown under conventional and organic cultural practices tended to have different phytochemical contents, but the results were inconsistent or unsubstantiated (Asami, Hong, Barrett, & Mitchell, 2003). Additionally, available studies comparing minerals in organically and conventionally grown beets are rare (Hansen, 1980; Pfiffner, Niggli, Velimirov, Boltzmann, Balzer, Balzer, et al., 1992). Therefore, a comprehensive study of mineral content and phytochemical profiles in both production systems is needed.

Comparing phytochemical profiles requires accurate methods for extracting and measuring phytochemicals. The nature of the extraction solvent, polarity, and solvent combination plays a crucial role in extracting the hydrophilic betalines and measuring their levels (Celli & Brooks, 2017). Among various extraction techniques, solid-liquid extraction is commonly performed for extracting natural pigments (Cardoso-Ugarte, Sosa-Morales, Ballard, Liceaga, & San Martín-González, 2014). Betalains are normally extracted from samples by different organic solvents such as methanol, ethanol, acetone, and mixtures of solvents (Fathordoobady, Mirhosseini, Selamat, & Manap, 2016; T. S. Kujala, Vienola, Klika, Loponen, & Pihlaja, 2002; Narkprasom, Su, Cheng, Wang, Hsiao, & Tsai, 2012); however, the reported levels of betalains and phytochemicals are

significantly different. Therefore, the present study was conducted to optimize the extraction efficiency phytochemicals using twenty different solvent compositions from red and golden beets grown in conventional and organic production systems. Further, HPLC and UPLC-QTOF-HR-MS were used to quantify and identify the phytochemicals present in extract. All the extracts were tested for total phenolics and radical scavenging activities to understand the effect of production system and extraction efficiency in each beet variety.

5.1. Material and Methods

5.1.1. Plant materials

Bunches of beetroots (*Beta vulgaris*) were purchased at the local Farm Patch and HEB grocery stores (College Station, TX, USA). Samples included conventionally and organically grown red and golden beets. The inedible parts such as the leaves and stems were removed, and the roots were washed with peel and wiped with paper towels. The beetroots were cut into cubes (1.5 ×1.5 ×1.5 cm) and processed in a 12-speed Oster blender (Sunbeam, FL, USA), then a known amount of each sample was weighed in separate 50-mL tubes and the rest of the paste was stored at -20°C.

5.1.2. Chemicals

L-ascorbic acid, gallic acid, formic acid, acetic acid, phosphoric acid, sodium carbonate, 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), Folin Ciocalteu (FC) reagent, 2, 2´-azinobis (3-etylbenzothiszoline-6-sulphonic acid) diammonium salt (ABTS), methanol, ethanol, LC-MS and HPLC grade acetonitrile, and crude betanin extract were obtained from Sigma-Aldrich

(St. Louis, MO, USA). All other reagents and solvents were analytical grade. Nanopure water (NANOpure, Barnstead, Dubuque, IA) was used for the entire study.

5.1.3. Sample preparation for mineral analysis

The cleaned beetroots were cut into pieces and freeze-dried (LabConco, Kansas City, MO, USA). The lyophilized samples (5 g) were submitted to Soil, Water, and Forage Testing Laboratory, Texas A&M University, College Station, TX in triplicate. Micronutrient quantification was conducted by inductively coupled plasma atomic emission spectroscopy (ICP-AES). First the beet samples were digested with nitric acid 125°C for 4 h and set aside at room temperature overnight. Then, the samples were diluted with distilled water and analyzed by ICP-AES.

5.1.4. Extraction of betalains from beetroots

Five grams of beetroot paste was extracted with 10 mL of solvent (see Table A-4) in a 50-mL tube by vortexing (30 s), homogenizing (1 min), and sonicating (1 h). Afterwards, the tubes were centrifuged at 800 × g for 15 min. The supernatant was collected, and the residue was extracted again with 3 mL of the same solvent. The supernatants were pooled, and the total volume was measured. Two mL of each was passed through a 0.45-μm membrane filter paper for HPLC and mass spectrometry analysis and the rest of the sample was stored -20°C for bioassays. All extractions were carried out in triplicate and the results were expressed as means and standard error.

5.1.5. Purification of betanin by flash chromatography

Pure betanin is not commercially available for quantification of betalains. Therefore, crude beet extract was loaded on a C18 glass reverse phase flash column (particle size 40–63 µm, 200 × 60 mm), (ACE Glass Inc, Vineland, NJ, USA). The separation of betanin was carried out on a flash chromatography system (Combiflash Rf, Teledyne Isco, Lincoln, NE, USA). Before loading the sample, the C18 column was equilibrated with 1 L of 1% aqueous formic acid for 30 min. Then, the separation of betanin was performed by a gradient program using solvent A (1% formic acid) and solvent B (acetonitrile) with a flow rate of 40 mL/min. The betalains were monitored at 480 and 540 nm with a peak width of 15 s and threshold of 0.05 AU. A total of 180 fractions of 15 mL each were collected. All fractions were analyzed by HPLC and fractions 48–56 containing the same peak were pooled and lyophilized to obtain pure compound. The purity and structure of betanin was confirmed by HPLC and UPLC-HR-MS.

5.1.6. Quantification the major betalains by HPLC

HPLC was performed using an Agilent 1200 HPLC series (Agilent Technologies, Palo Alto, CA, USA) connected to a photodiode array detector. The separation was carried out on a Gemini C_{18} Column (250 mm × 4.6 mm i.d.) (Phenomenex, Torrance, CA, USA) at 30°C. The mobile phase consisted of 0.3 M phosphoric acid (solvent A) and acetonitrile (solvent B). At a flow rate of 0.8 mL/min, different betalains were monitored at 540, 480, and 420 nm and identified by comparing retention times and UV spectra from published papers (Slatnar, Stampar, Veberic, & Jakopic, 2015b). Different concentrations (31.25–500 µg/mL) of purified betanin were injected into the HPLC and a calibration curve was prepared. Samples of 10 µL were injected and the betalain content was expressed as µg betanin equivalents/g of fresh weight.

5.1.7. Total phenolics, DPPH, and ABTS radical scavenging activity

5.1.7.1. Estimation of total phenolics

The total phenolic content was assessed using the Folin-Ciocalteu assay according to our previous publication (G. K. Jayaprakasha, Girennavar, & Patil, 2008). The absorbance was measured at $\lambda = 734$ nm and total phenolics were expressed as µg gallic acid equivalents/g of fresh weight.

5.1.7.2. Estimation of total phenolics

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay was conducted according to our published paper (Bae, Jayaprakasha, Jifon, & Patil, 2012) and results were expressed as µg ascorbic acid equivalents/g of fresh weight.

5.1.7.3. ABTS radical scavenging activity

A slight modification of the published method (G. K. Jayaprakasha, Girennavar, & Patil, 2008) was used to assess the 2,2´-azinobis-(3-ethylbenzotiazoline-6-sulphonic acid) radical scavenging activity. Results were expressed as µg ascorbic acid equivalents/g of fresh weight.

5.1.8. Identification of phytochemicals by UHPLC-QTOF-MS

Ultra-high-performance liquid chromatography (UHPLC) separation was performed with the 1290 Agilent HPLC LC system (Agilent Technologies, Santa Clara, CA) equipped with a maXis impact mass spectrometer (Bruker Daltonics, Billerica, MA, USA). An Eclipse Plus rapid resolution C_{18} column (1.8 µm, 50 × 2.1 mm) was used for the separation of phytochemicals with

a binary mobile phase consisting of 0.2% formic acid in water (A), and 0.2% formic acid in acetonitrile (B). The temperature of the autosampler compartment was set at 35°C. The flow rate was 0.5 mL/min. The gradient program consisted of 0% B (0–2 min), linear gradient from 0% B to 35% B (2–9 min), then increased to 100% B (9–14 min) and back to 0% B (14–16 min). The post-run equilibration time was 2 min. The total run time was 18 min.

For quadrupole time-of-flight mass spectrometry (QTOF-MS), the maXis Impact mass spectrometer was operated in positive electrospray ionization mode and was equipped with a six-port divert valve for calibration. MS and bbCID data (line spectra) were acquired in the m/z range of 50–1200. The capillary voltage of the ion source was 4,200 V. The nebulizer (nitrogen) gas pressure was 4.0 bar and the drying gas flow rate 12.0 L/min. The drying gas temperature was 250°C. The transfer time of the source was 120.8 μs and the prepulse storage time 1 μs. The quadrupole MS collision energy and bbCID collision energy were set at 5 and 55 eV, respectively. The spectra rate was 1.4 Hz. In bbCID, the precursor ions were fragmented in the collision cell without pre-selection. By alternating the acquisition between MS and bbCID conditions, high and low collision energy data sets were collected simultaneously. External instrument calibration was performed with a sodium formate solution containing 1 mM sodium hydroxide in isopropanol with 0.2% formic acid (1:1 v/v). Nine sodium formate clusters were used in the calibration using the high-precision calibration mode. An automated post-run internal mass scale calibration of individual samples was performed by injecting the above calibrant at the end of each run. Calibration of each sample was performed at the end of the run.

5.1.9. Statistical and chemometric analysis

The significance of variation in the levels of minerals were analyzed by one-way analysis of variance (ANOVA) by employing JMP Pro12 software (SAS, NC, USA). Results were expressed as mean value ± standard error. A probability of 5% or less was accepted as statistically significant. Multivariate data analysis was performed by translating the LC-MS data in csv format and subjecting to the online MetaboAnalyst software 4.0 (https://www.metaboanalyst.ca/).

5.2. Results and Discussion

5.2.1. Differences in mineral contents of conventional and organic beets

In the present study, we measured the relative levels of minerals in red and golden beets from conventional and organic production systems (Table A-5). Significantly higher levels of some minerals (N, P, Mg, Na, Fe, Mn, and B) were found in conventional red beets, whereas organic beets had higher levels of K, Ca, and Cu, but the difference in Zn was non-significant. In golden beets, the conventionally produced beets had higher accumulation of all minerals except P and N, which were higher in the organic beets. There are no comprehensive data correlating the effects of conventional and organic production systems on mineral levels of beetroots (Hansen, 1980; Pfiffner, et al., 1992). One study found that Mg and Cu were higher in organically grown beets than in conventionally grown beets. However, variation in the levels of P and K were dependent on the growing locations (Hansen, 1980). Pfiffner and coauthors found K was higher in conventional beets, but the P, Ca, Mg contents fluctuated each year. Therefore, genetic, agronomic, and environmental factors could significantly influence the content and composition of minerals (Rouphael, Cardarelli, Bassal, Leonardi, Giuffrida, & Colla, 2012).

5.2.2. Identification of phytochemicals from beetroots by UHPLC-QTOF-MS

The individual constituents of the samples obtained by extracting with twenty solvents were assessed by UHPLC-QTOF-MS. Twenty-seven phytochemicals including 23 betalain compounds, 2 amino acids, and 2 phenolic acids, were identified using high resolution mass spectral data. The retention time, molecular formula, molecular mass, and MS/MS fragments of individual compounds are presented in Table 7.

Betalains include betacyanins and betaxanthins. Betacyanins are condensed products of betalalamic acid and have various substitutions (glycosylation or acylation) of one or both hydroxyl groups at position 5 or 6 of the betanidin (Belhadj Slimen, Najar, & Abderrabba, 2017). In red beetroot varieties, the main betacyanin peaks were identified as betanin (m/z 551), the simplest 5-O-glucosylated betacyanin, and its C-15 isoform isobetanin (m/z 551) with the fragment ion at m/z 389 (betanidin). Compared to the organic red beet, conventional red beet extracts had the more betalains, which included acylated betacyanins such as 2′-O-glucosyl-betanin (m/z 713), 6′-feruloyl betanin (m/z 727), and 6′-feruloyl-isobetanin (m/z 727). These were modified through the bond of the acyl group to the sugar moiety (glucose, glucuronic acid, and apiose) via an ester linkage and had two contiguous glucosyl groups attached to the C5 carbon, and the 2,17-decarboxylzed betacyanin compounds, which were 17-decarboxy-(iso) betanidin (m/z 345), 17-decarboxy-betanin (m/z 507), 2,17-decarboxy-neobetanin (m/z 505), 2,17-bidecarboxy-2,3-dehydro-neobetanin (m/z 459), and 2-decarboxy-2,3-dehydro-neobetanin (m/z 503). Betanidin (m/z 389) and 17-decarboxy-isobetanidin (m/z 345) were not detected in the organic beetroot extracts. Consistent with previous research, betacyanins were not detected at significant levels in conventional and organic golden beetroots (Slatnar, Stampar, Veberic, & Jakopic, 2015b).

In contrast to betacyanins, betaxanthins react with free amino acids and form new compounds with amino acid adducts. In golden beet extracts, we identified betaxanthins with glutamine, valine, leucine, and phenylalanine adducts. These betaxanthin derivatives were also present in conventional and organic red beet extracts. In addition, other amino acids, phenylalanine (m/z 166) and L-tryptophan (m/z 205), were identified based on their molecular mass and MS/MS fragmentation. Further, hydroxy caffeic acid (m/z 197) and syringic acid (m/z 199) were present in four beet cultivars. Previous studies identified these two phenolic acids in beetroot samples (Nemzer, et al., 2011; Obata, Senba, & Koshika, 1963).

5.2.3. Variation in betalains in conventional and organic red and golden beets measured by HPLC

The total betalains of red and golden beet extracts are shown in Table A-6 and the levels were varied from 1138- 4039 µg/g fresh weight (FW). The highest amount of total betalains was detected in the ethanol: water extract (S6), followed by methanol extract (S13), and the lowest amount was in the water: ascorbic acid extract (S2). Similar concentrations of total betalains were reported in previous study of 13 red beet varieties from Poland (Sawicki, Bączek, & Wiczkowski, 2016), where the total betalains of red beet were in the range of 1.57–2.70 mg/g FW, and the amount significantly differed depending on the beet variety and also extraction solvent. In comparison, red beets grown in Australia had less total betalains with contents of 0.38–0.65 mg/g FW (Wruss, et al., 2015).

In organic red beet extracts, we observed higher variation compared to conventional red beet extracts, with the organic beets having 0.5–12.3 mg/g FW betalain. Similar to the results for conventionally grown red beets, the total betalain concentration was high in the methanol extract

(S13), and the lowest amount was detected in the water extract (S1). Furthermore, the beetroot extracted with water with or without acid gave a lower yield of betalains, compared to the organic solvents. This may be due to the mucilaginous effect of water-soluble pectin compounds (Fathordoobady, Mirhosseini, Selamat, & Manap, 2016). Hence, water may not be a useful solvent for the extraction of betalains from organic red beet.

Our results were consistent with the literature, which illustrated that betalain contents might be influenced by several factors, including varietal, growing season, and climatic and cultivation conditions (Sawicki, Bączek, & Wiczkowski, 2016). In addition, the average amount of total betalains were significantly higher in 18 organic red beet extracts than in their conventional counterparts, except for extracts obtained from water (S1) and ethanol : water : acetic acid (S11), which had 4-fold and 1-fold less betalains than the conventional red beet extracts. This result indicated that the production system also affect the betalain contents, which could be partly explained by the difference in nitrogen levels (Nemzer, et al., 2011)

Significant variations of total betalains were also found in golden beet extracts, wherein the total betalains varied from 0.0–0.8 mg/g FW for conventionally grown golden beets, and 0.0–1.3 mg/g FW for organic golden beets. Conventional golden beets extracted by methanol: water : ascorbic acid (S17) had the highest total phenolics. By contrast, betalain levels were not detectable in the water (S1), water/formic acid (S2), ethanol: water：acetic acid or methanol ： water ： acetic acid mixture (S11 and S19), and methanol：water ： formic acid (S20) extracts. In organic golden beet extracts, the highest total betalains were found in the methanol ： water ： formic acid extract (S20). To the best of our knowledge, little information regarding the content of betalains in different beet extracts has been reported so far.

In general, total betalains were higher in red beet than in golden beet, with amounts being 3-fold higher in conventionally grown red beets, and 8-fold higher in organically grown cultivars. This finding was in accordance with previous research, where total betalain content in red beetroot was significantly higher than that in yellow beetroot, regardless of the extraction solvent (Sawicki, Bączek, & Wiczkowski, 2016). The higher amount of betalains in red beet indicate that the limited utilization of tyrosine, the precursor of betalain synthesis, occurs in yellow beetroot compared to the red beetroot. In the biosynthetic pathway of betalains, tyrosine is first oxidized to L-dihydroxy phenylalanine (L-DOPA) by reacting with CYP76AD1, AD5, and AD6 L-DOPA is then converted to betalamic acid and *cyclo*-DOPA, which are required for betalain production. Betalamic acid and *cyclo*-DOPA are needed for betacyanin productions, whereas only betalamic acid along with amino acids or amines are required for betaxanthins. The limited amounts of tyrosine in golden beet may cause the overall low betalain content (M. Wang, Lopez-Nieves, Goldman, & Maeda, 2017). To verify this, peak areas were generated for betalain biosynthesis-related metabolites (Fig 9). Indeed, the levels of tyrosine were much higher in conventionally and organically grown red beets than in golden beets. As a result, the levels of L-DOPA (m/z 198) were higher, and the *cyclo*-DOPA (m/z 196) and betalamic acid (m/z 212) were higher, ultimately causing higher betalain levels in red beet than golden beets. This was consistent with previously reported findings (Slatnar, Stampar, Veberic, & Jakopic, 2015b; M. Wang, Lopez-Nieves, Goldman, & Maeda, 2017), showing that the primary and secondary metabolites within the betalain biosynthesis pathway can be influenced by the variety of vegetable.

The levels of betanin and vulgaxanthin I quantified by HPLC were expressed as betanin equivalents (Table 6), since vulgaxanthin I is not commercially available. The conventional red beet extracted with methanol： water： formic acid (S20) had the highest betanin content (2791.0

μg/g FW), and the water： ascorbic acid extract (S2) had the lowest betanin (578.8 μg/g FW). The isobetanin was highest in the water： acetic acid (S4) extract, 5–9 fold higher than in other water-containing solvents (S1, S2, S3). Interestingly, conventional red beet extracted by aqueous methanol mixed with organic acids (S17, S18, S19) had higher betanin contents than the other methanol solvent extract (S16). This result is in agreement with Narkprasom and others, who demonstrated that organic acids enhanced betalain retention through oxygen scavenging and complexation of metal cations (Narkprasom, Su, Cheng, Wang, Hsiao, & Tsai, 2012). Another study indicated that slight acidification of the extraction medium enhances betacyanin stability and avoids oxidation by polyphenol oxidases (Schliemann, Kobayashi, & Strack, 1999).

In the present study, the highest vulgaxanthin I and valine-betaxanthin content was found in the ethanol： water extract (S6, 1260.6 and 193.9 μg/g FW). In organic red beet samples, the highest betanin and vulgaxanthin I content was found in the methanol extract (8222.3 and 4031.5 μg/g FW), and the water extract had the lowest betanin (364.2 μg/g FW). In comparison, the highest isobetanin was found in the ethanol： water： formic acid (S12) extract (549.5 μg/g FW), followed by methanol： water： formic acid (S18).

With respect to conventionally grown golden beet, the highest vulgaxanthin I content was detected in samples extracted by methanol： water： ascorbic acid (S17, 746.1 μg/g FW); however, no valine-betaxanthin was found in this extract. The highest valine-betaxanthin was found in the aqueous methanol (S16) extract, with a content of 80.9 μg/g FW. Organic golden beet extracted by aqueous ethanol (S6, 836.2 μg/g FW) had the highest vulgaxanthin I content compared to other solvents. Whereas in water extract, no vulgaxanthin I was detected. Organic golden beet extracted by aqueous ethanol S8 had the highest valine-betaxanthin content (104.6 μg/g FW), suggesting that aqueous ethanol was efficient at extracting betaxanthins. We note here

that betalains, betacyanins, and betaxanthins were measured by UV spectroscopy due to the lack of a pure standard, whereas we purified betanin as a standard and carried out HPLC analysis, which proved to be a suitable method for separation of betalains (Stintzing, Schieber, & Carle, 2002b).

5.2.4. Influence of solvent combination on total phenolics and radical scavenging activities

Figure 7 shows the results of total phenolics as well as DPPH and ABTS free radical scavenging activities for the samples from conventional and organic red beets. The application of diverse solvent ratios and combinations resulted in beet extracts with significantly different total phenolics and DPPH and ABTS activities. Conventional red beetroot extracted by methanol alone (S13) had the highest phenolics (323.67 µg gallic acid equivalents [GAE]/g FW). A previous study (Boeing, Barizão, e Silva, Montanher, de Cinque Almeida, & Visentainer, 2014) reported that methanol was an effective solvent due to the interactions between the polar sites of the antioxidant molecules and the solvent. The lowest total phenolics value was found in the methanol ： water extract (S16, 159.57 µg GAE/g FW), which indicated that the ratio of methanol significantly affects the extraction of total phenolics. Interestingly, the conventional red beet extracted by aqueous methanol solvents with added ascorbic, formic, or acetic acid had lower total phenolics than the methanol alone (S13, 191.21 to 292.16 µg GAE/g FW), suggesting that acidification might increase the recovery of phenolic compounds in red beet. However, this trend was not observed in the ethanol extracts of red beetroot. The highest total phenolics of organic red beetroot was obtained from the ethanol ： water (S8) extract (436.69 µg GAE/g FW), and the lowest total phenolics was found in the water ： acetic acid (S4) extract (153.64 µg GAE/g FW). Other water-containing solvent (S1, S2, S3) extracts showed moderate amounts of total phenolics.

Among the various extracts of golden beets (Fig. 8), conventionally grown golden beetroot extracted by pure ethanol (S13) had the highest total phenolics (212.35 µg GAE/g FW), and the lowest amount was found in the methanol： water： formic acid extract (S18, 35.59 µg GAE/g FW). In contrast to conventionally grown red beet, the water extract of conventionally grown golden beet had high total phenolics. Significant variation in total phenolics was observed in organic golden beet extracts, with the highest value found in the methanol： water： ascorbic acid extract (S15, 123.55 µg GAE/g FW), and the lowest in the methanol： water：acetic acid extract (S19, 27.54 µg GAE/g FW). Interestingly, total phenolics in conventional and organic golden beets were higher in the extracts with solvents containing ascorbic acid than those with formic or acetic acid, suggesting that ascorbic acid acidified solvents could be the potential favorite solvent for extracting health-promoting compounds in golden beet.

DPPH and ABTS assays were performed to evaluate the *in vitro* antioxidant activities of the different beet extracts. The highest DPPH and ABTS radical scavenging activities were found in aqueous methanol： formic acid (S18) extracts, with ascorbic acid (AA) equivalents of 400.76 and 229.83 µg AA/g FW, respectively. The lowest DPPH and ABTS activities were found in the methanol： ascorbic acid (S17) extract (91.64 and 88.01 µg/AA g FW). In comparison, organic red beet extracted with methanol： water： formic acid (S20), and methanol： water： acetic acid (S19) had the highest DPPH activity (453.50 and 376.44 µg/AA g FW). The lowest DPPH (104.92 µg/AA g FW) and ABTS (44.03 µg/AA g FW) activities were found in water: ascorbic acid (S4), and ethanol: water: ascorbic acid (S9) extracts respectively. Consistent with the total phenolics results, the overall DPPH and ABTS activities of organic red beet extracted by organic solvents were significantly higher than the samples extracted by water solvents. In some of the ethanol-containing solvents, conventional and organic red beet ethanol： water (S6), ethanol：

water：formic acid (S10), and ethanol：water：acetic acid (S11) extracts, high DPPH and ABTS activities were present. Given their high extraction efficiency and lower toxicity compared with other solvents, aqueous ethanol combined with acid might be considered as a desirable replacement for methanol.

For conventionally grown golden beet, the ethanol：water：formic acid (S10) extract had the highest DPPH activity (164.04 µg AA/g FW) and the ethanol：water extract (S6) had the lowest DPPH activity (37.16 µg AA/g FW). Consistent with the total phenolics results, the highest ABTS activity was detected in the ethanol extract (S5, 96.57 µg AA/g FW). However, moderate DPPH inhibitory activity was found in this extract, consistent with the differing reaction mechanisms in the two assays. This was consistent with the DPPH and ABTS results in organic golden beet extracts, wherein the water with acid extract exhibited high ABTS but low DPPH scavenging activities.

The overall DPPH and ABTS radical scavenging activities of red beetroot were significantly higher than golden beetroot. In our present results, betanin was the predominant betacyanin in red beetroot, whereas vulgaxanthin I was the main betaxanthin in golden beetroot. Betanin contains an aromatic ring with the betalamic acid moiety, a second ring combined with an indoline, which increases the radical scavenging activity. The antiradical activities of betanin and its isomer were further enhanced by the additional hydroxyl group in their benzene rings (Fig. 10) (Gandía-Herrero, Escribano, & García-Carmona, 2010). In comparison, vulgaxanthin I was the main betalain in golden beet, and had minimal antiradical activities due to its structure lacking aromatic resonance, charged, or hydroxyl groups. Therefore, the radical scavenging activities of golden beetroot were t significantly lower compared to red beets.

5.2.5. Chemometrics-based approach to identify the influence of extraction solvent on phytochemical profiles

In the present study, multivariate statistical analysis was applied to build an intuitive overview of the beet extract results. The abundances of phytochemicals identified by LC-MS are presented as a heatmap in Fig.10. Significant variations in phytochemicals were detected in conventional red beet extracted with the 20 solvents. Among these, beets extracted by water ： ascorbic acid (S2), ethanol (S5), different concentration of ethanol with ascorbic acid (S7 and S9), and methanol : water : formic acid (S20) had higher amounts of betacyanin derivatives, namely betanidin (m/z 389), 6′-feruloyl-betanin and its isomer (m/z 727), prebetanin (m/z 631), betanin (m/z 551), 2′-glucosyl-betanin (m/z 713), 2,17-bidecarboxy-2,3-dehydro-neobetanin (m/z 459), 17-decarboxy-neobetanin (m/z 505), neobetanin (m/z 549), 17-decarboxy-betanidin (m/z 345), and amino acid-adducted betaxanthins including valine-betaxanthin (m/z 311), leucine-betaxanthin (m/z 325), proline-betaxanthin (m/z 309), phenylalanine-betaxanthin (m/z 359), and glutamic acid-betaxanthin (m/z 341).

Higher levels of betanin, one of the main betacyanins in red beet, was found in aqueous ethanol ： ascorbic acid extracts (S7 and S9). Another main compound, isobetanin (m/z 551), was highest in water ： formic acid (S3) extracts, with the mass area 1- to 430-fold higher than the conventional red beet extracted with other water-containing solvents (S1–3). In addition, amino acids such as phenylalanine (m/z 166), L-tryptophan (m/z 205), and the phenolic acid syringic acid (m/z 199), were also identified in those extracts. Two decarboxylated betacyanins, 17-decarboxy-betanin (m/z 507) and 2-decarbocy-neobetanin (m/z 505), were found at the highest levels in the aqueous ethanol ： formic acid (S10) extract, with an area 1 to 3-fold higher than the ethanol：

acetic acid (S11) extract. Another compound, 2-decarboxy-2,3-dehydro-neobetanin (m/z 503) had a significantly higher area in the aqueous ethanol (S8) extract. The water : ethanol solvent system affects the stability of betanin, since ethanol has a high electron density on the oxygen atom, leading to nucleophilic attack on the oxygen atom, and ultimately causing decarboxylation (Azeredo, 2009).

Conventional red beet extracted by water or aqueous combined with ascorbic acid solvents (S2, S7, S9) obtained larger quantities of phytochemicals compared to the extracts obtained from formic : acetic acid (S3, S4) or aqueous ethanol (S6), indicating ascorbic acid with water or ethanol might be an effective solvent combination for obtaining large quantities of betalains. The overall different abundance of betalains in beet extracts may be attributed to their interaction with the extracting solvents, which altered the linear symmetry restrictions of the betanin molecule and thus changed their characteristics (Thankappan, Thomas, & Nampoori, 2013). Similarly, in organic red beet extracts, water and ascorbic mixture (S2), ethanol with or without ascorbic acid (S5, S7, and S9), and methanol : water : formic acid (S20) solvents were efficient in obtaining various betalain compounds such as decarboxylated betacyanins, namely 2-decarboxy-neobetanin (m/z 505), 17-decarboxy-neobetanin (m/z 505), 17-decarboxy-betanidin (m/z 345), 2-decarboxy-2,3-dehydro-neobetanin (m/z 503), and 2,17-bidecarboxy-2,3-dehydro-neobetanin (m/z 459). In water/formic acid (S3) extract, and ethanol : water : acid (S10, S12) extracts, only one betacyanin with relative high levels was found. For instance, isobetanin (m/z 551) was identified in water : formic acid extract (S3) with a mass area 4-fold higher than water extract (S1), and 17-fold higher than methanol : water : formic acid extract (S20). In other solvent combinations (S1, S8, S13, S15-19), the levels of phytochemicals were negligible. However, betalains include betanidin (m/z 389) and 17-decarboxy-isobetanidin (m/z 345), glutamic acid-betaxanthin (m/z

341), leucine-betaxanthin (m/z 325), and phenolic acids, hydroxy caffeic acid (m/z 197) and syringic acid (m/z 199), were not detected in all organic red beet extracts, suggesting that those compounds might be considered as biomarkers to discriminate conventional and organic red beets.

The conventional golden beet samples extracted by methanol (S13), aqueous ethanol and ascorbic acid mixtures (S7 and S9) were rich in L-tryptophan (m/z 205) and valine-betaxanthin (m/z 311). Phenylalanine (m/z 166) was detected in extracts obtained by water with or without formic/acetic acid (S1, S3 and S4) extracts, aqueous methanol with ascorbic acid mixture (S15 and S17) were detected. Similar to conventional red beet, γ-aminobutyric acid-betaxanthin (m/z 297) was also detected in S15 and S17 extracts. Glutamine-isobetaxanthin (m/z 340) was only detected in ethanol, water, and acetic acid extract (S11) with high levels. In other extracts, this compound was not detected. Proline-betaxanthin (m/z 309) was detected in ethanol (S5) and aqueous methanol (S14) extracts. Other solvents, which were S2, S6, S8, S18-20 were absent in those compounds.

In organic golden beet, the methanol, water, and formic acid mixture (S20) solvent extract namely leucine-betaxanthin (m/z 325), glutamine-betaxanthin (m/z 340), γ-aminobutyric acid-betaxanthin (m/z 297) exhibited the highest mass areas, with the levels 19 to 91-folds, 1 to 49-folds, and 14 folds higher than in aqueous ethanol or methanol extracts, respectively. The glutamine-betaxanthin (m/z 340) was only detected in ethanol, water, and ascorbic acid (S7) extract. However, few amino acids were found in aqueous ethanol and methanol extracts (S6 and S16). The other extracts contained only low levels of those phytochemicals.

5.3. Conclusion

The present study indicated that solvent combination significantly influences the composition and content of phytochemicals in beet samples. UHPLC-QTOF-MS analysis combined with chemometrics identified 27 phytochemicals of which 27, 21, 6, and 6 phytochemicals were in conventional red, organic red, conventional golden, and organic golden beets, respectively. The levels of these compounds differed based on each solvent composition. Distinct compounds identified from heatmap analysis might be considered as potential biomarkers to discriminate beet extracts obtained from various solvents or raw beets from different production systems. Conventional red beet extracted by S18 showed the highest DPPH and total phenolics, and methanol extract (S13) had the highest total phenolics.

In organic red beet, aqueous ethanol extract with or without acid (S8 and S19) had the highest total phenolics, DPPH, and ABTS values. Conventional and organic golden beet extracted by ethanol (S7 and S10) had higher antioxidant activities than other extracts. This provides useful information for optimizing methods for identification of phytochemicals and biomarkers.

Table 6. Levels of betanin, isobetanin, vulgaxanthin I, and valine-betaxanthin in beet extracts.

	Conventional Red				Organic Red				Conventional Golden				Organic Golden			
No.	Betanin	Isobetanin	Vulgaxanthin I	Valine-betaxanthin	Betanin	Isobetanin	Vulgaxanthin I	Valine-betaxanthin	Betanin	Isobetanin	Vulgaxanthin I	Valine-betaxanthin	Betanin	Isobetanin	Vulgaxanthin I	Valine-betaxanthin
S1	2116.1 ± 39.5	82.8 ± 2.6	694.1 ± 14.7	97.2 ± 5.1	364.2 ± 21.1	14.7 ± 0.6	113.7 ± 8.9	31.9 ± 0.8	ND	ND	320.1 ± 7.6	ND	ND	ND	ND	ND
S2	578.8 ± 19.1	56.4 ± 0.8	454.0 ± 5.5	49.1 ± 1.4	2861.1 ± 93.9	77.0 ± 2.1	2125.2 ± 280.5	191.4 ± 8.5	ND	ND	456.7 ± 29.9	27.5 ± 1.1	ND	ND	289.4 ± 13.0	ND
S3	1693.8 ± 5.5	95.4 ± 1.2	121.9 ± 4.2	58.7± 1.6	3219.8 ± 105.9	93.2 ± 2.0	597.9 ± 10.6	80.6 ± 1.5	ND	ND	ND	39.0 ± 2.0	ND	ND	30.8 ± 3.0	ND
S4	2758.3 ± 91.9	601.8 ± 24.5	618.2 ± 15.4	58.6 ± 1.2	3270.5 ± 142.6	116.2 ± 1.3	159.7 ± 18.3	313.3 ± 16.4	ND	ND	47.4 ± 2.6	ND	ND	ND	9.6 ± 0.3	ND
S5	835.1 ± 80.5	39.6 ± 2.8	232.1 ± 9.5	61.3 ± 1.5	3199.4 ± 149.1	94.4 ± 3.0	851.0 ± 5.8	162.6 ± 3.2	ND	ND	230.5 ± 77.0	30.5 ± 2.0	ND	ND	148.1 ± 1.2	10.5 ± 0.2
S6	2545.3 ± 42.8	39.1 ± 1.9	1260.6 ± 8.1	193.9 ± 3.4	3862.6 ± 60.1	77.3 ± 3.5	922.4 ± 34.3	193.7 ± 5.4	ND	ND	491.8 ± 7.4	22.6 ± 6.3	ND	ND	836.2 ± 5.0	18.8 ± 1.8
S7	1321.6 ± 21.4	73.7 ± 1.3	471.4 ± 4.8	65.6 ± 0.7	3752.6 ± 30.6	115.9 ± 3.4	1673.5 ± 29.4	218.3 ± 5.3	ND	ND	348.9 ± 6.8	19.7 ± 0.9	ND	ND	363.5 ± 7.4	23.6 ± 0.7
S8	2472.0 ± 62.3	84.7 ± 4.8	885.4 ± 30.7	75.2 ± 4.9	3583.1 ± 26.1	103.2 ± 2.1	1752.1 ± 7.8	207.6 ± 2.6	ND	ND	461.1 ± 26.4	33.5 ± 0.7	ND	ND	234.4 ± 25.0	104.6 ± 9.2
S9	1516.3 ± 6.0	74.5 ± 1.6	545.1 ± 2.7	83.6 ± 2.2	2544.9 ± 23.4	62.8 ± 3.6	1180.3 ± 1.0	214.8 ± 2.3	ND	ND	448.1 ± 14.7	24.4 ± 1.5	ND	ND	529.0 ± 3.0	44.6 ± 0.9
S10	2328.3 ± 24.6	146.8 ± 1.2	460.6 ± 7.7	65.3 ± 1.7	3019.9 ± 156.9	264.8 ± 13.8	662.3 ± 41.6	122.0 ± 7.6	ND	ND	54.3 ± 0.9	48.1 ± 2.0	ND	ND	62.4 ± 0.5	6.4 ± 0.2
S11	2712.9 ± 24.8	121.0 ± 3.3	775.9 ± 7.4	68.9 ± 0.7	1725.0 ± 46.9	62.8 ± 1.7	581.6 ± 21.7	80.6 ± 1.9	ND	ND	ND	18.3 ± 0.5	ND	ND	17.4 ± 2.3	ND
S12	1149.0 ± 11.5	106.6 ± 3.1	228.3 ± 10.5	83.4 ± 2.9	3575.9 ± 74.1	549.5 ± 15.9	380.6 ± 7.8	170.8 ± 6.0	ND	ND	93.5 ±11.9	ND	ND	ND	131.1 ± 15.4	17.7 ± 3.6
S13	2448.8 ± 21.8	89.3 ± 2.7	1133.6 ± 7.4	162.1 ± 2.0	8222.3 ± 471.6	163.8 ± 14.0	4031.5 ± 212.3	67.9 ± 4.1	ND	ND	659.4 ± 4.2	7.5 ± 0.2	ND	ND	701.9 ± 30.5	16.6 ± 2.6
S14	1421.2 ± 8.3	53.4 ± 1.6	535.6 ± 4.3	63.6 ± 1.2	2546.2 ± 76.8	75.0 ± 2.7	1391.4 ± 59.4	153.3 ± 4.9	ND	ND	428.7 ± 15.8	17.3 ± 0.7	ND	ND	423.9 ± 37.4	19.0 ± 0.5
S15	1071.6 ± 58.2	51.5 ± 2.5	385.6 ± 22.9	46.9 ± 4.1	5479.4 ± 243.1	186.3 ± 8.9	2085.8 ± 63.9	179.3 ± 5.7	ND	ND	699.7 ± 26.4	24.2 ± 1.3	ND	ND	363.5 ± 7.4	23.6 ± 0.7
S16	1287.3 ± 39.1	42.7 ± 0.8	499.0 ± 7.5	75.6 ± 1.9	3542.8 ± 63.5	85.7 ± 2.4	1758.0 ± 4.0	157.4 ± 3.8	ND	ND	235.5 ± 20.3	80.9 ± 2.8	ND	ND	588.5 ± 25.7	29.0 ± 2.2
S17	2014.1 ± 26.4	65.6 ± 1.8	638.9 ± 16.4	97.8 ± 3.8	2621.2 ± 141.8	134.0 ± 4.6	358.6 ± 25.3	109.8 ± 9.6	ND	ND	746.1 ± 4.3	ND	ND	ND	710.5 ± 28.1	45.4 ± 1.9
S18	2791.0 ± 60.2	220.7 ± 7.8	335.3 ± 17.9	62.6 ± 2.1	3776.8 ± 214.6	460.0 ± 12.4	556.8 ± 29.7	83.0 ± 2.6	ND	ND	120.4 ± 6.9	70.3 ± 2.8	ND	ND	83.2 ± 7.1	9.6 ± 0.4
S19	2335.7 ± 22.4	76.9 ± 1.7	511.2 ± 9.0	44.1 ± 1.9	4727.2 ± 20.8	109.9 ± 1.2	1505.7 ± 26.2	75.6 ± 4.5	ND	ND	ND	12.7 ± 1.9	ND	ND	24.6 ± 3.8	11.1 ± 2.2
S20	1453.7 ± 7.2	53.9 ± 1.8	595.5 ± 8.2	71.7 ± 3.9	8183.9 ± 67.5	154.8 ± 4.9	3507.0 ± 13.9	78.2 ± 2.7	ND	ND	320.1 ± 7.6	ND	ND	ND	ND	ND

Table 7. Identified phytochemicals found in conventionally and organically grown red and golden beets.

No.	Identified compounds	RT (min)	Molecularformula	Experimentalmass	Exactmass	Mass error (ppm)	MS/MS fragments	Beet Varieties				Reference
								Con Red	Org Red	Con Golden	Org Golden	
1	Glutamine-isobetaxanthin	1.30	$C_{14}H_{17}N_3O_7$	340.1172	340.1139	9.7	321, 277	x	x	x	x	(Nemzer, et al., 2011)
2	Glutamine-betaxanthin	1.55	$C_{14}H_{17}N_3O_7$	340.1172	340.1139	9.7	321, 277	x	x		x	
3	Phenylalanine	3.15	$C_9H_{11}NO_2$	166.0877	166.0863	8.4	-	x	x	x		(Goodban, Stark, & Owens, 1953)
4	Glutamic acid-betaxanthin	3.50	$C_{14}H_{16}N_2O_8$	341.1009	341.0979	8.8	215	x				(Nemzer, et al., 2011)
5	Hydroxy caffeic acid	3.85	$C_9H_8O_5$	197.0431	197.0445	-7.1	-	x				
6	17-Decarboxy-betanidin	4.15	$C_{17}H_{17}N_2O_6^+$	345.1109	345.1081	8.1	-	x	x			
7	17-Decarboxy-isobetanidin	4.25	$C_{17}H_{17}N_2O_6^+$	345.1102	345.1081	6.1	-	x				
8	γ-Aminobutyric acid-betaxanthin	4.65	$C_{13}H_{16}N_2O_6$	297.1103	297.1081	7.4	-	x	x	x	x	
9	Proline-betaxanthin	4.80	$C_{14}H_{16}N_2O_6$	309.1107	309.1081	8.4	283, 195	x	x		x	
10	L-tryptophan	5.15	$C_{11}H_{22}N_2O_2$	205.0986	205.0972	6.8	-	x	x	x	x	
11	Prebetanin	5.35	$C_{24}H_{26}N_2O_{16}S$	631.1127	631.1076	8.1	551, 389	x	x			
12	Betanin	5.45	$C_{24}H_{26}N_2O_{13}$	551.1511	551.1508	0.5	389, 150	x	x			
13	2´-O-glucosyl-betanin	5.65	$C_{34}H_{37}N_2O_{15}^+$	713.2086	713.2189	-14.4	551, 389	x	x			
14	Isobetanin	5.85	$C_{24}H_{26}N_2O_{13}$	551.1502	551.1508	-1.1	389, 150	x	x			
15	Betanidin	6.35	$C_{18}H_{16}N_2O_8$	389.1016	389.0979	9.5	343	x				
16	Valine-betaxanthin	6.50	$C_{14}H_{18}N_2O_6$	311.1263	311.1238	8.0	-	x	x	x		
17	17-Decarboxy-betanin	6.75	$C_{22}H_{26}N_2O_{11}$	507.1723	507.1609	22.5	345	x	x			
18	Syringic acid	6.95	$C_9H_{10}O_5$	199.0590	199.0601	-5.5	-	x				(Obata, Senba, & Koshika, 1963)
19	Neobetanin	7.05	$C_{24}H_{24}N_2O_{13}$	549.1389	549.1352	6.7	387	x	x			(Nemzer, et al., 2011)
20	17-Decarboxy-neobetanin	7.05	$C_{23}H_{24}N_2O_{11}$	505.1565	505.1453	22.2	343, 297	x	x			
21	2-Decarboxy-neobetanin	7.10	$C_{23}H_{24}N_2O_{11}$	505.1568	505.1453	22.8	343, 297	x	x			
22	2,17-Bidecarboxy-2,3-dehydro-neobetanin	7.90	$C_{22}H_{22}N_2O_9$	459.1501	459.1398	22.4	-	x	x			
23	Leucine-betaxanthin (Vulgaxanthin IV)	8.00	$C_{13}H_{15}N_3O_7$	325.1419	325.1394	7.7	-	x			x	
24	Phenylalanine-betaxanthin	8.25	$C_{18}H_{18}N_2O_6$	359.1261	359.1238	6.4	313			x		
25	2-Decarboxy-2,3-dehydro-neobetanin	8.60	$C_{23}H_{22}N_2O_{11}$	503.1336	503.1296	8.0	341	x	x			
26	6´-feruloyl-betanin	9.40	$C_{34}H_{35}N_2O_{16}^+$	727.2053	727.1981	9.9	453, 389		x			
27	6´-feruloyl-isobetanin	9.60	$C_{34}H_{35}N_2O_{16}^+$	727.2044	727.1981	8.7	453, 389		x			

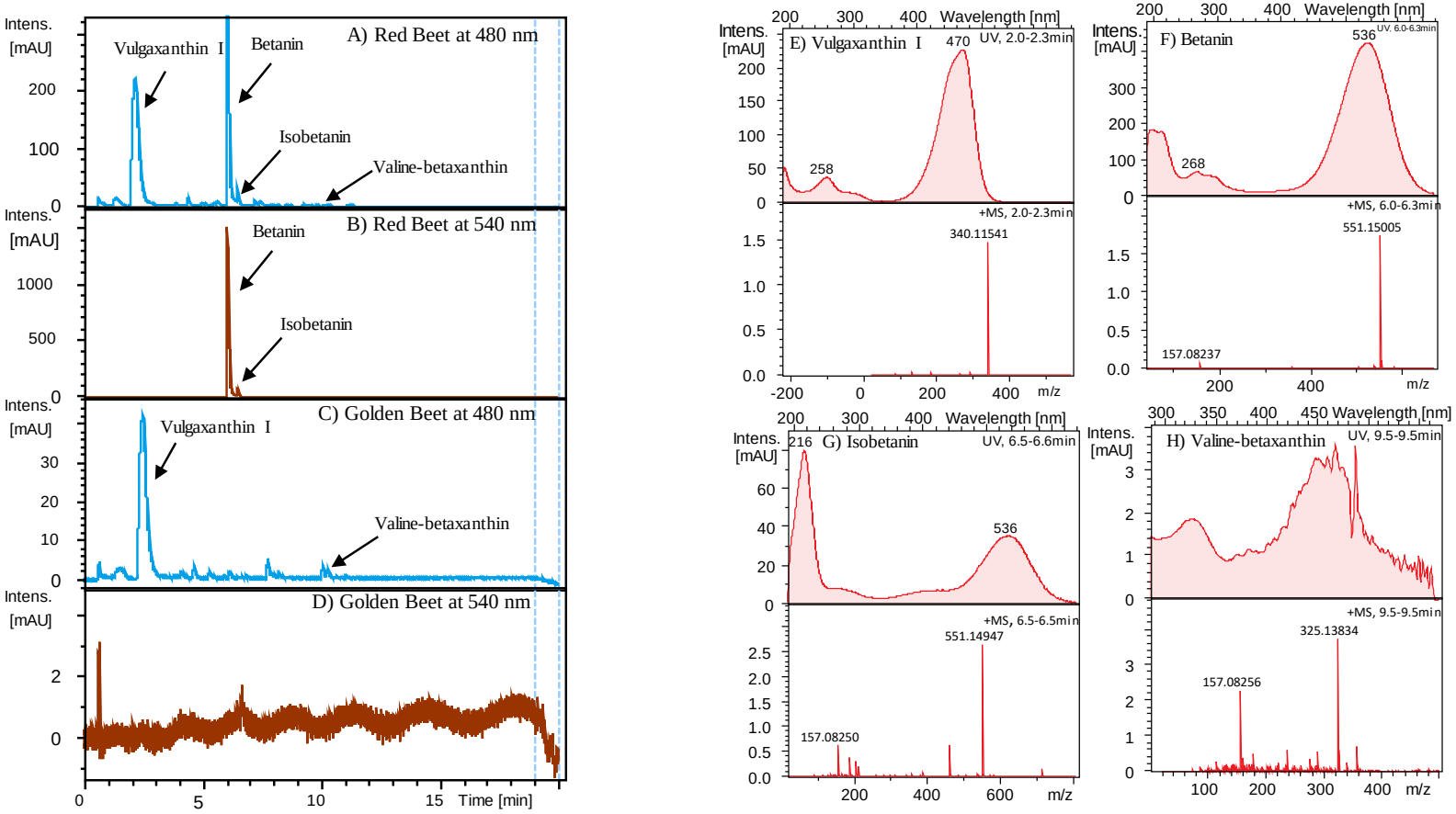

Figure 6. UV and UPLC chromatograms of (A, B) red beet and (C, D) golden beet, with mass spectra of betalains of (E–H) red beet and (G–H) golden beet obtained by HR-ESI-QTOFMS analysis in positive ionization mode.

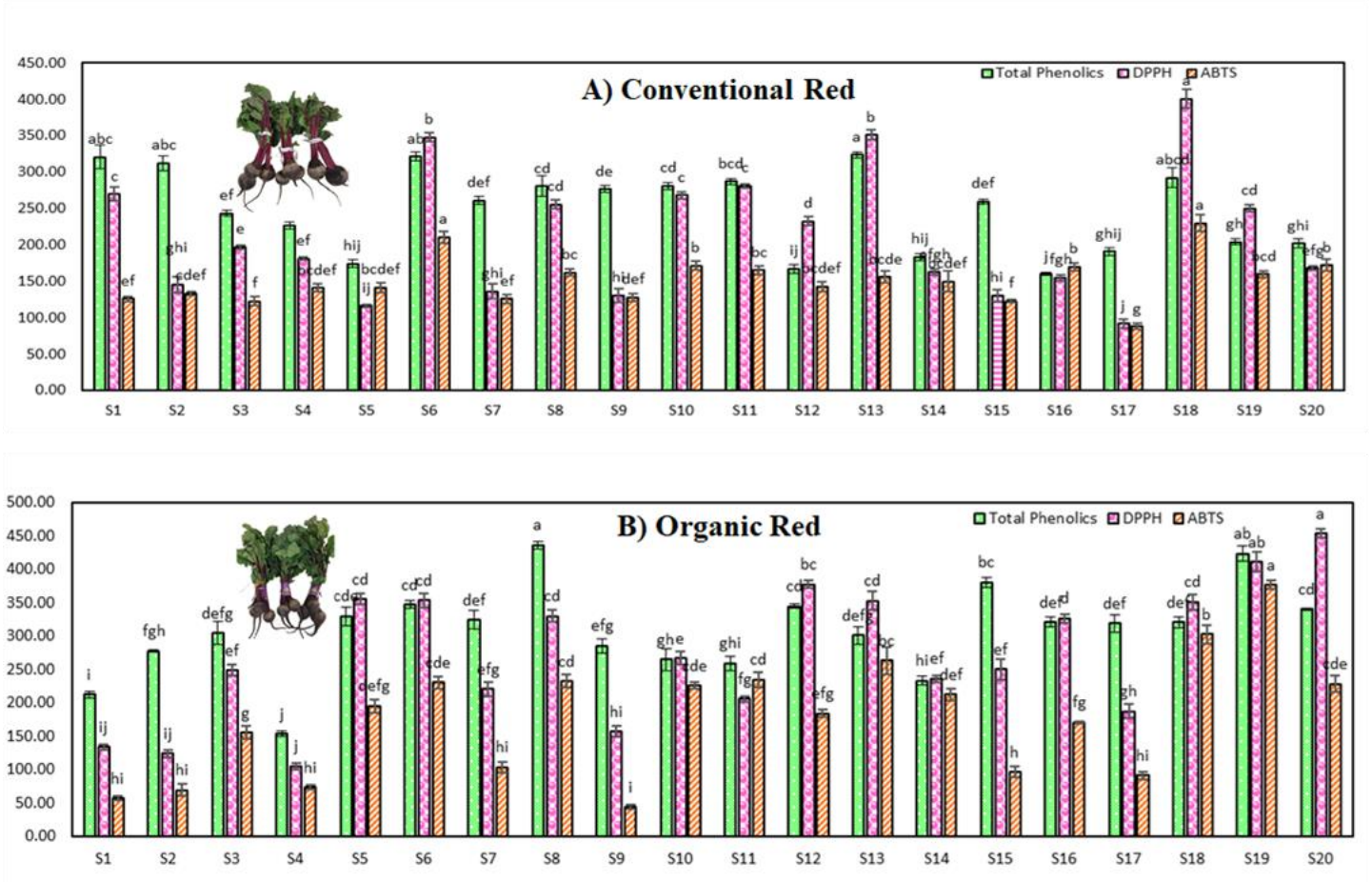

Figure 7. Total phenolics, plus DPPH and ABTS radical scavenging activity of (A) conventional and (B) organic red beets extracted with twenty different solvent combinations (see Table S1). Data for total phenolics are expressed as µg gallic acid equivalents per g of fresh weight. Data for DPPH and ABTS are expressed as µg ascorbic acid equivalents per g of fresh weight. X axis: solvent code, Y axis: ascorbic acid equivalents. Values were expressed as mean ± SE. Different letters indicate significant differences at $P \leq 0.05$.

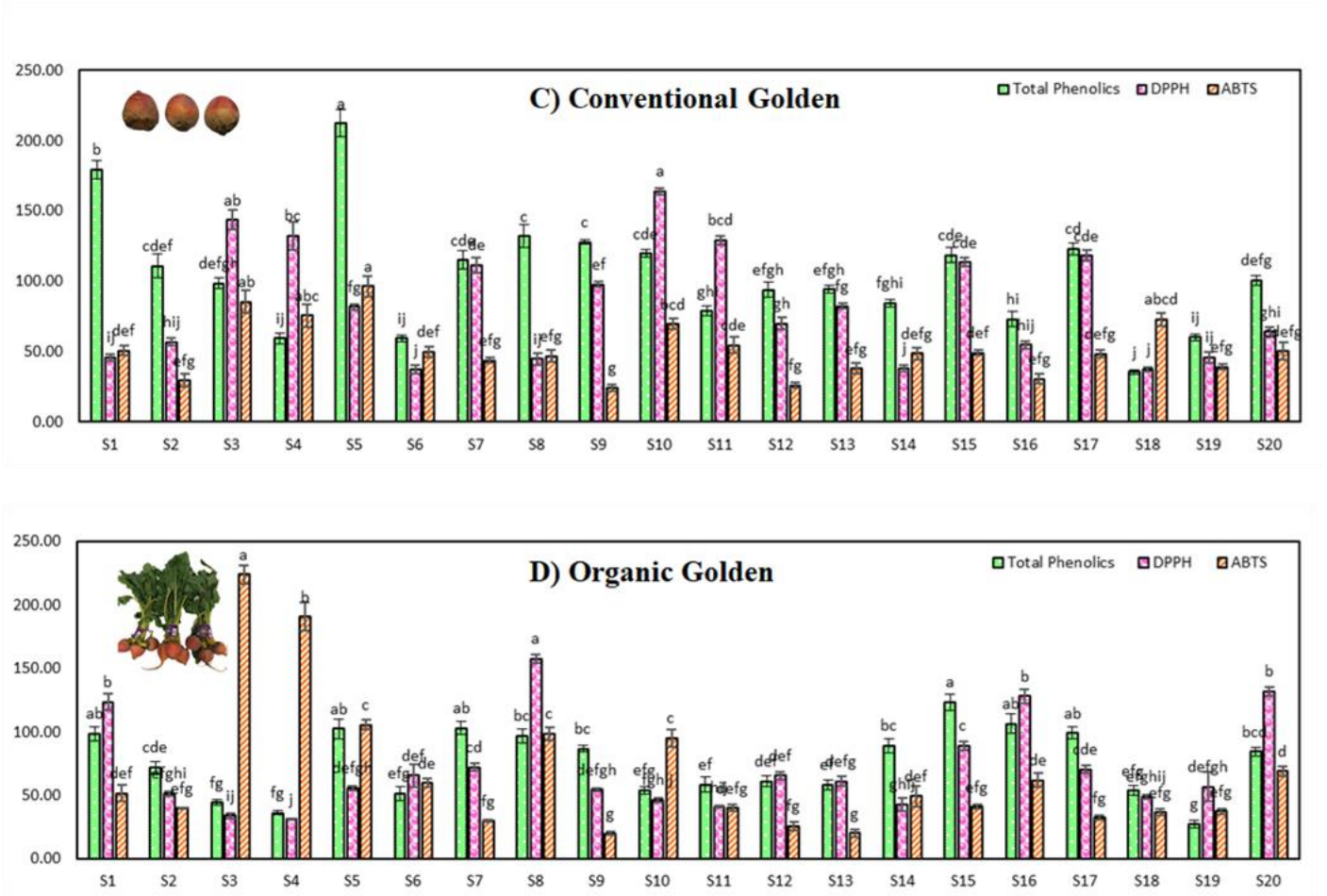

Figure 8. Total phenolics, plus DPPH and ABTS radical scavenging activity of (A) conventionally and (B) organically grown golden beets extracted with twenty solvent combinations (see Table S1). Data for total phenolics expressed as µg gallic acid equivalents per g of fresh weight. Data for DPPH and ABTS expressed as µg ascorbic acid equivalents per g of fresh weight. X axis: solvent code, Y axis: ascorbic acid equivalent values. Values were expressed as mean ± SE. Different letters indicate significant differences at $P \leq 0.05$.

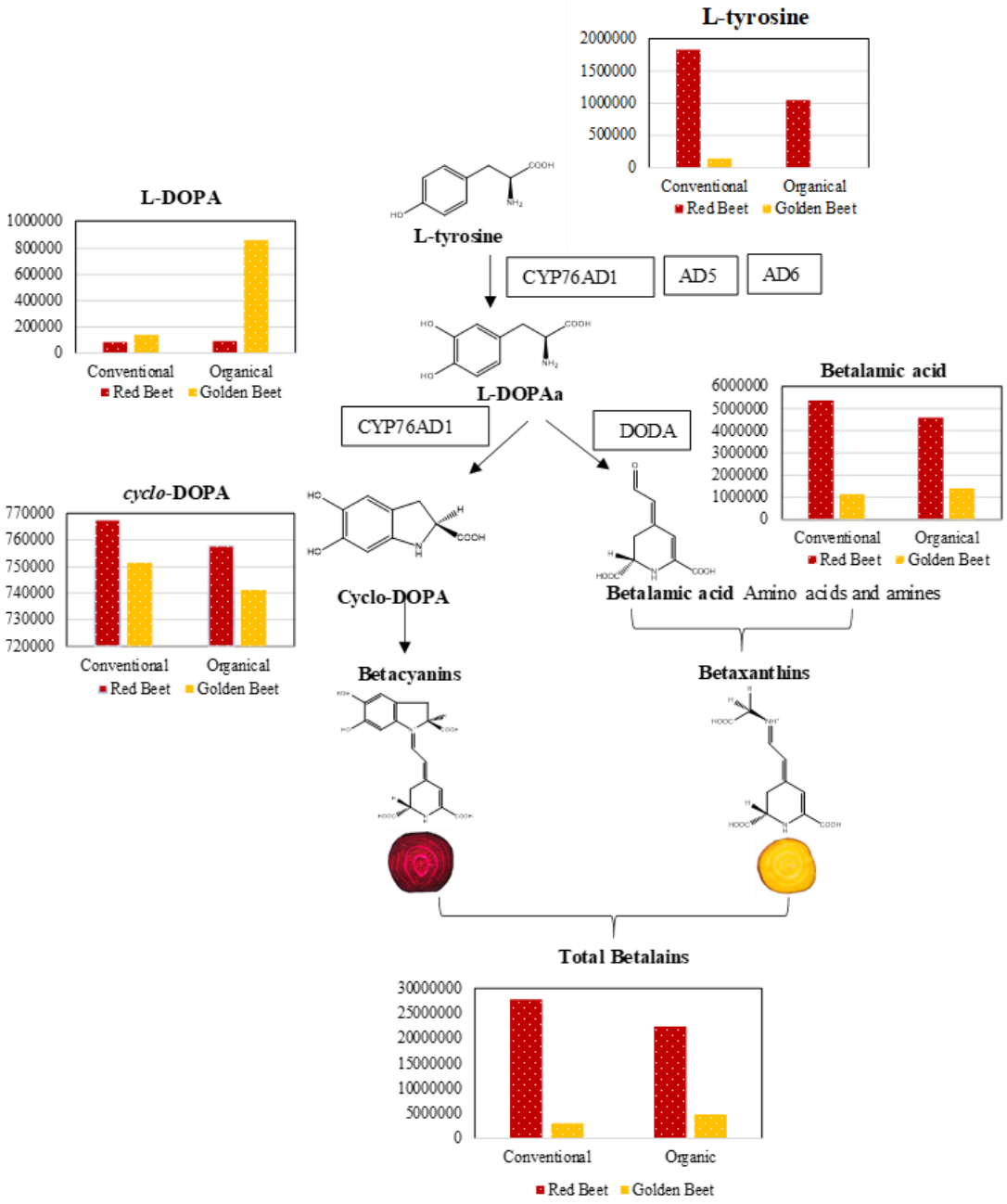

Figure 9. Betalain biosynthetic pathway and relative contents of main metabolites in conventionally and organically grown red and golden beet extracts. The *y* axis represents the sum of peak areas for each compound. Modified from (M. Wang, Lopez-Nieves, Goldman, & Maeda, 2017).

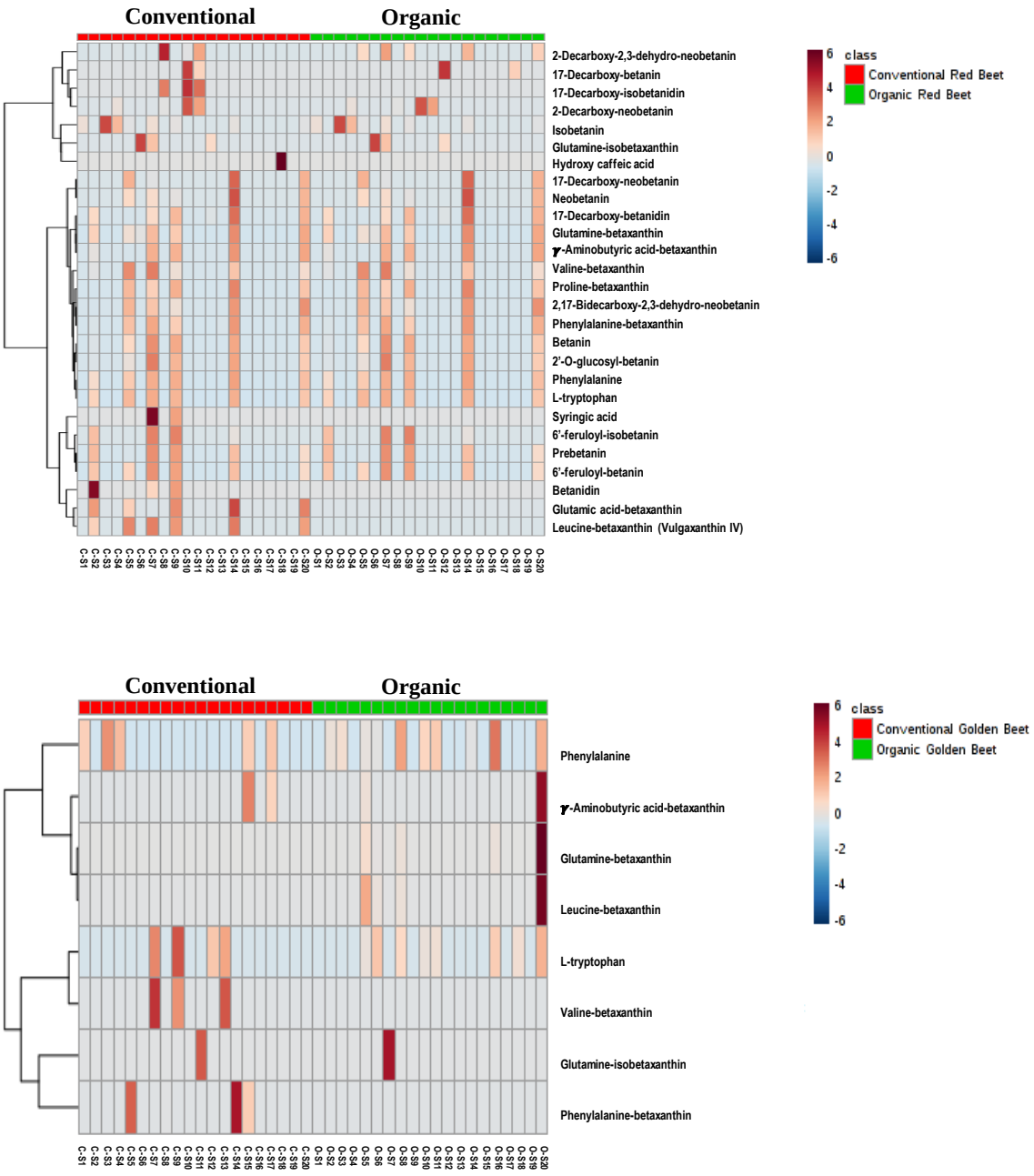

Figure 10. Heatmap of metabolites from conventionally and organically grown (A) red beet and (B) golden beet grown extracted by 20 solvents (see Table S1) based on Pearson distances and Ward clustering. With O: organic, C: conventional. Colored squares represent the abundance of each compound with blue indicating the lowest values and red the highest values.

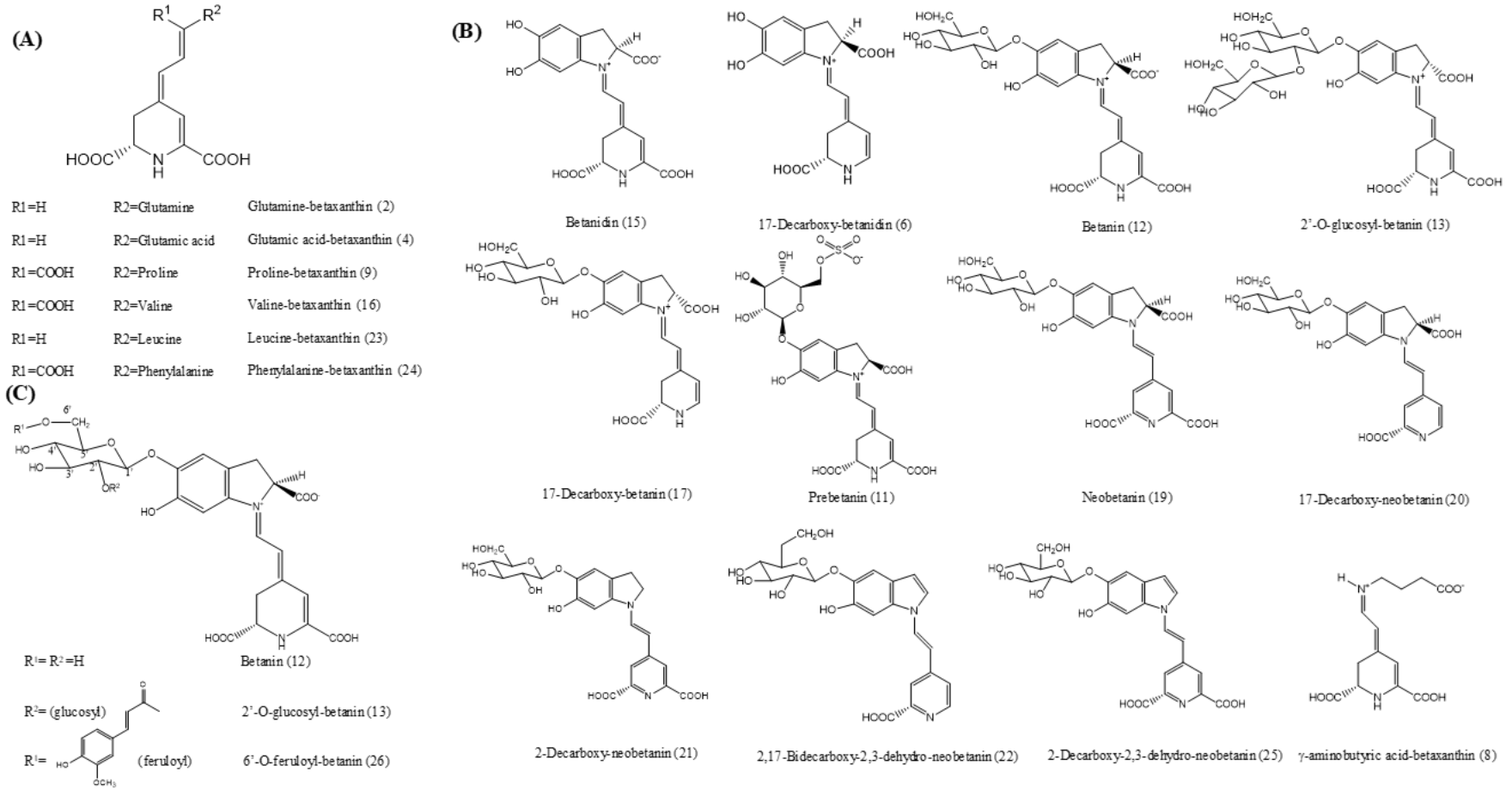

Figure 11.Chemical structures of (A) betaxanthins and (B, C) betacyanins present in conventional and organic beets. The number in parenthesis of each compound matches Table 7.

6. SUMMARY AND CONCLUSION

Fruits and vegetables contain a wealth of phytochemicals. Accumulative studies have reported the relevance of regular consumptions of these phytochemicals to maintain health or reduce the risk of chronic diseases. Therefore, a comprehensive study concerns the effects of domestic and industrial processing and extraction on the phytochemicals of the fruits, vegetables, and their processed products is needed to provide the overall metabolite profile and assist food manufacturers optimize the products with desirable sensorial and nutritional balance. In the present study, a range of targeted and non-targeted metabolomic platforms, combined with chemometric approaches are applied to investigate the impact of processing techniques, production system, and extraction solvents on the metabolites profile and antioxidant activities in the fruits, vegetables, and their processed products. The first study deployed the three processing techniques (blending, high-speed centrifugal juicing and low-speed juicing) to unravel their effects to phytochemical profiles in 21 vegetables. Kaempferol and quercetin glycosides, decarboxylated betalains, and quercetin derivatives were found to be representative metabolites in separating the processing techniques and production system in the respective kale, beet, and melon juices. The effects of three processing techniques (blending, high-speed centrifugal juicing, and low-speed juicing) on phytochemical profiles and antioxidant activities were investigated by using UHPLC–QTOF-MS coupled to complementary targeted and untargeted metabolomics approaches. The results exhibited 73 different phytochemicals belonging to various chemical classes, such as flavonoids and betalains, and four novel betalains. Combined with chemometric principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), characteristic metabolites such as kaempferol glycosides and amino acid attached betaxanthins were chosen for discriminating the three processing techniques between kale and beet extracts, with the low-speed juicing retains

more kaempferol glycosides in *Brassica* vegetables, whereas the high-speed centrifugal juicing acquires more betalains. The antioxidant activities were significantly different in juices based on the three processing techniques and vegetable varieties, with the purple baby carrot blended juice had the highest total phenolics (717.67 µg/g GAE) and DPPH value (715.77 µg/g AA). This research provides a thorough insight for manufacturers in optimizing the process techniques to produce high-standard vegetable products.

The second study investigated the phenolic fingerprinting by using untargeted metabolites analysis through UPLC-QTOF-MS combined with chemometrics to identify potential metabolites by discriminating the 30 commercially obtained thermally and cold processed juices. HPLC results indicated all juices were desirable sources of phytochemicals, with the varied ascorbic acid (3-661 µg/mL) and total ascorbic acid (7-1183 µg/mL) content. The pure beet juice had the highest nitrate content (1075 µg/mL). Antioxidant activity results demonstrated that food ingredients and processing technique massively affected the results, in which the thermally processed kale, beet, and melon juices exhibited higher total phenolics and antioxidant activities than the cold-pressed juices. The results demonstrated that chemometrics is an efficient tool for discriminating thermally and cold-pressed commercial juices.

The present study assessed twenty solvent mixtures containing water, methanol, and ethanol alone or combined with an acid (ascorbic, formic, acetic) for extraction efficiency and antioxidant activities. These were tested on conventionally or organically grown red and golden beets (*Beta vulgaris*). Red beet extracted with methanol with or without acid had the highest betanin content (2791.0 µg/g and 8222.3 µg/g of fresh weight [FW]), and the water extracts had the lowest betanins (578.8 µg/g and 364.2 µg/g of FW). Golden beet extracted with methanol: ascorbic acid: water had the highest vulgaxanthin I (193.7 µg/g and 15.0 µg/g of FW). Tests of

radical-scavenging activity and total phenolics in beet extracts reflected the different extraction efficiency of each solvent. Ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) identified 25 phytochemicals in conventional red, 20 in organic red, 6 in conventional golden, and 6 in organic golden beet extracts, respectively. The untargeted metabolomics combined chemometrics discriminated the beet varieties and different extracts within on variety based on the composition and abundance of the key phytochemicals, which were decarboxylated betacyanins in red beet (conventional and organic), and amino acid adducted betaxanthins in golden beet (conventional and organic) extracts, separately.

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APPENDIX A

TABLES FOR ADDITIONAL INFORMATION

Table A-1. Commercial name, scientific name, yield, and color measurement of 21 vegetables processed by three juicing techniques.

Scientific name	Name (color)	ocessing	Yield (%)	L*	a*	b*
Brassica oleracea var botrytis	Cauliflower (W)	Oster Blender	93.1	73.62 ± 0.40	0.23 ± 0.40	26.16 ± 0.36
	Cauliflower (W)	Breville Juice Extractor	35.1	77.08 ± 0.39	-5.68 ± 0.11	25.32 ± 0.16
	Cauliflower (W)	Omega Juicer	35.6	80.15 ± 0.71	-3.17 ± 0.04	19.76 ± 0.55
	Cauliflower (Y)	Oster Blender	88.2	71.61 ± 0.38	8.13 ± 0.11	47.17 ± 0.49
	Cauliflower (Y)	Breville Juice Extractor	27.8	74.11 ± 0.78	5.99 ± 0.38	46.92 ± 0.81
	Cauliflower (Y)	Omega Juicer	29.6	70.31 ± 0.65	6.11 ± 0.88	49.02 ± 1.38
	Cauliflower (G)	Oster Blender	92.1	60.49 ± 0.40	-9.04 ± 0.01	31.72 ± 0.80
	Cauliflower (G)	Breville Juice Extractor	29.8	74.93 ± 0.22	12.79 ± 0.10	39.95 ± 0.48
	Cauliflower (G)	Omega Juicer	31.2	74.03 ± 0.02	10.01 ± 0.10	31.23 ± 0.32
Brassica oleracea var. sabellica	Kale (G)	Oster Blender	94.3	33.90 ± 0.01	-6.40 ± 0.08	12.38 ± 0.19
	Kale (G)	Breville Juice Extractor	17.6	45.81 ± 0.95	17.21 ± 0.95	28.54 ± 2.38
	Kale (G)	Omega Juicer	36.8	36.41 ± 0.27	10.31 ± 0.08	11.70 ± 0.16
	Kale (GO)	Oster Blender	92.3	30.43 ± 0.34	-3.82 ± 0.05	4.23 ± 0.06
	Kale (GO)	Breville Juice Extractor	33.7	32.57 ± 0.17	-6.53 ± 0.14	7.98 ± 0.28
	Kale (GO)	Omega Juicer	42.8	31.14 ± 0.37	-6.53 ± 0.12	7.22 ± 0.27
	Kale (RO)	Oster Blender	92.7	30.78 ± 0.34	-2.87 ± 0.13	5.99 ± 0.53
	Kale (RO)	Breville Juice Extractor	35.9	29.16 ± 0.77	-1.39 ± 0.89	3.06 ± 1.15
	Kale (RO)	Omega Juicer	55.0	28.87 ± 0.82	-0.65 ± 0.33	1.43 ± 0.28
	Kale (BO)	Oster Blender	94.6	29.27 ± 0.18	-4.86 ± 0.05	5.01 ± 0.59
	Kale (BO)	Breville Juice Extractor	27.2	30.57 ± 0.08	-6.22 ± 0.07	5.49 ± 0.12
	Kale (BO)	Omega Juicer	52.3	29.41 ± 0.64	-5.38 ± 0.38	5.47 ± 0.56
	Turnip (P)	Oster Blender	93.1	59.7 ± 0.08	9.35 ± 0.12	21.49 ± 0.14
	Turnip (P)	Breville Juice Extractor	44.9	78.74 ± 1.13	0.28 ± 0.22	11.52 ± 1.38
	Turnip (P)	Omega Juicer	48.6	77.81 ± 0.38	0.34 ± 0.18	9.84 ± 1.19
	Turnip (W)	Oster Blender	79.4	68.44 ± 0.31	2.78 ± 0.04	25.99 ± 0.15
	Turnip (W)	Breville Juice Extractor	52.1	79.45 ± 0.88	-2.17 ± 0.07	12.67 ± 1.00
	Turnip (W)	Omega Juicer	47.0	82.85 ± 1.02	-0.77 ± 0.08	8.65 ± 0.95
Raphanus raphanistrum subsp. sativus	Radish (R)	Oster Blender	92.0	54.30 ± 0.53	17.81 ± 0.48	19.3 5± 0.32
	Radish (R)	Breville Juice Extractor	50.6	66.96 ± 2.52	15.69 ± 1.04	5.78 ± 0.21
	Radish (R)	Omega Juicer	53.3	80.19 ± 0.24	9.54 ± 0.38	2.63 ± 0.09
	Radish (RO)	Oster Blender	93.6	50.28 ± 1.33	31.13 ± 0.98	7.28 ± 0.76
	Radish (RO)	Breville Juice Extractor	52.9	63.24 ± 1.05	20.08 ± 0.35	4.67 ± 0.19
	Radish (RO)	Omega Juicer	56.6	67.36 ± 2.17	15.86 ± 1.63	3.97 ± 0.71
	Radish (G)	Oster Blender	85.8	35.53 ± 0.07	25.77 ± 0.17	8.43 ± 0.09
	Radish (G)	Breville Juice Extractor	50.1	47.81 ± 2.53	43.74 ± 1.44	4.44 ± 0.77
	Radish (G)	Omega Juicer	51.5	46.55 ± 0.52	42.84 ± 0.61	4.05 ± 0.46
	Radish (W)	Oster Blender	87.7	72.57 ± 0.72	-1.74 ± 0.01	17.17 ± 0.27
	Radish (W)	Breville Juice Extractor	56.0	84.14 ± 1.58	-0.42 ± 0.06	3.85 ± 0.15
	Radish (W)	Omega Juicer	63.2	85.46 ± 0.77	-0.17 ± 0.09	3.53 ± 0.71

Table A-1. Continued

Scientific name	Name (color)	ocessing	Yield (%)	L*	a*	b*
Beta vulgaris	Beet (R)	ter Blender	92.0	52.55 ± 0.21	7.74 ± 0.16	29.46±0.05
	Beet (R)	eville Juice Extractor	45.4	71.31 ± 0.72	-4.11 ± 0.52	44.58±1.20
	Beet (R)	nega Juicer	53.7	75.81 ± 0.58	-5.90 ± 0.14	38.72±0.87
	Beet (RO)	ter Blender	93.6	48.40 ± 0.45	21.92 ± 0.27	31.85±0.33
	Beet (RO)	eville Juice Extractor	37.6	60.45 ± 0.82	32.03 ± 0.94	47.07±1.98
	Beet (RO)	nega Juicer	39.4	62.00 ± 0.72	29.94 ± 1.18	50.01±1.06
Daucus carota subsp. sativus	Beet (G)	ter Blender	91.4	32.47 ± 0.18	2.03 ± 0.01	12.72 ± 0.09
	Beet (G)	eville Juice Extractor	37.8	34.25 ± 1.16	6.72 ± 0.63	17.11 ± 1.77
	Baby Carrot (O)	nega Juicer	44.3	62.00 ± 0.72	29.94 ± 1.18	50.01±1.06
	Baby Carrot (P)	ter Blender	88.8	29.20 ± 0.07	5.19 ± 0.16	2.59±0.14
	Baby Carrot (P)	eville Juice Extractor	38.1	25.75 ± 0.98	6.45 ± 0.17	-2.20±0.34
	Baby Carrot (P)	nega Juicer	37.9	24.83 ± 1.40	5.42 ± 0.63	-2.10±0.17
	Baby Carrot (Y)	ter Blender	76.9	46.09 ± 0.32	8.95 ± 0.14	30.45±0.28
	Baby Carrot (Y)	eville Juice Extractor	43.9	62.44 ± 1.66	9.32 ± 0.34	52.19±0.24
	Baby Carrot (Y)	nega Juicer	33.7	59.62 ± 0.67	10.47 ± 0.31	51.79±1.27
	Baby Carrot (W)	ter Blender	91.5	52.55 ± 0.21	7.74 ± 0.16	29.46±0.05
	Baby Carrot (W)	eville Juice Extractor	52.0	71.31 ± 0.72	-4.11 ± 0.52	44.58±1.20
	Baby Carrot (W)	nega Juicer	40.4	75.81 ± 0.58	-5.90 ± 0.14	38.72±0.87

Color: W: white, Y: yellow, G: green, GO: green organic, BO: black organic, P: purple, WB: white baby, R: red, RO: red organic; G: golden, GO: golden organic. L*: brightness; a*: redness; b*yellowness; C*: Chroma; h: hue angle.

Table A-2. Thirty thermal, cold, and high-pressure processed juices categorized as kale (K1–K10), beet (B1–B10), and melon (M1–10) juices*

Code	Juice Name (Brand)	Processed by	Ingredients Present in the Juice	Volume (mL)	Price/Bottle ($)	Price/Oz ($)
K1	Green Machine (Naked)	Thermal	Kale, apple and pineapple juice, mango, kiwi and banana puree, spirulina, natural flavors, alfalfa, broccoli, spinach, barley grass, wheatgrass, parsley, ginger root, odorless garlic	450	2.68	0.176
K2	Healthy Greens (V8)	Thermal	Spinach, kale, cucumber, celery, romaine lettuce, green bell pepper, pineapple, apples, yellow carrots, huito juice, watermelon juice, vitamin C	1360	2.87	0.062
K3	Grown Vegetable Juice (Estate)	Cold	Tomatoes, carrots, celery, kale, spinach, lime juice, beets, parsley, onions, coriander, watercress, basil, sea salt, garlic, oregano, thyme, pepper	354	3.49	0.291
K4	Green Delight Organic (Suja)	Cold	Kale, organic apple, lemon, spinach juice, organic banana mango puree, organic spirulina, chlorella, barley grass powder	354	2.98	0.248
K5	Uber Greens Organic (Suja)	Cold	Organic cucumber, celery, grapefruit, green chard, leaf lettuce, lemon, kale, spinach, parsley juice, Tea (peppermint, spearmint)	354	2.98	0.248
K6	Radiant Probiotic Organic (Suja)	Cold	Kale, celery, cucumber, fennel, apple, collard greens, lemon, spinach juice, ground cayenne pepper, Tea (peppermint, spearmint), probiotic *Bacillus coagulans*	354	2.98	0.248
K7	Organic Kale (1915)	Cold	Kale, organic apple, romaine lettuce, cucumber, spinach, lemon juice	354	3.99	0.333
K8	Organic Emerald greens (Evolution)	Cold	Kale, organic cucumber, celery, lemon, ginger, lime, spinach juice	325	3.99	0.363
K9	Organic Green Devotion (Evolution)	Cold	Kale, organic cucumber, celery, spinach, romaine lettuce, lemon, parsley juice	325	3.99	0.363
K10	Super Fruit Greens (Evolution)	Cold	Kale juice, organic orange, apple, pineapple, cucumber, spinach, romaine lettuce, spirulina, chlorella	325	3.99	0.363

Table A-2. Continued

Code	Juice Name (Brand)	Processed by	Ingredients Present in the Juice	Volume (mL)	Price /Bottle ($)	Price/Oz ($)
B1	Strawberry Banana (V8)	Thermal	Beet, apple, carrot, sweet potato, strawberry, banana, grapes, additives (citric acid, beta carotene, vitamin C, sucralose)	1360	2.87	0.062
B2	Original 100% Vegetables juice (V8)	Thermal	Beet, tomato, carrot, celery, beets, parsley, lettuce, watercress, additives (salt, vitamin C, natural flavoring, citric acid)	1360	2.44	0.062
B3	Original 100% Vegetables Juice Low Sodium (V8)	Thermal	Beets, tomato, carrot, celery, parsley, lettuce, watercress, additives (potassium chloride, salt, vitamin C, natural flavoring, citric acid)	340	0.88	0.077
B4	Spicy Hot 100% Vegetables Juice (V8)	Thermal	Beets, tomato, carrot, celery, parsley, lettuce, watercress, spinach, additives (salt, vitamin C, natural flavoring, citric acid)	1360	2.44	0.053
B5	Beet Juice (Biotta)	Thermal	Organic beetroot juice (lacto fermented)	500	5.49	0.325
B6	Organic Pure Beet (Lakewood)	Thermal	Organic beetroot juice,organic lemon juice (1%)	946	4.95	0.155
B7	Farms Daily Roots (Bolthouse)	Thermal	Beet, purple carrot, cucumber, purple sweet potato, lemon, monk fruit juice from concentrate, red bell pepper puree, parsley, lettuce, watercress, spinach, juice, vitamin (C, A, B6, B9, B12,) zinc, iron, magnesium, potassium, sea salt, natural flavor	450	2.97	0.195
B8	Very Veggie (R.W. Knudsen)	Thermal	Water, tomato paste, organic carrot and celery juice, organic grain vinegar, organic lemon juice concentrate, organic parsley and beet green, bell peppers, lettuce, watercress and spinach juice	946	2.49	0.078
B9	Organic Beet (1915)	Cold	Organic beet, carrot, orange, lemon juice	354	3.99	0.333
B10	Organic Cucum Berry (Simple Truth)	Cold	Organic cucumber, apple, beet, lemon juice, organic strawberry, banana, puree	354	3.99	0.333

Table A-2. Continued

Code	Juice Name (Brand)	Processed by	Ingredients Present in the Juice	Volume (mL)	Price/ Bottle ($)	Price/Oz ($)
M1	Synergy Watermelon Wonder (GTS)	Thermal	Organic kombucha culture, black tea, green tea, kiwi juice, fresh-pressed watermelon and lime juice, cherry juice	473	2.97	0.186
M2	Antioxidant Infusion (Bai)	Thermal	Water, erythritol, stevia leaf extract, natural flavors, citric acid, watermelon juice concentrate, vegetables juice, coffee fruit extract, white tea extract, malic acid, vitamin C, sodium citrate	530	1.50	0.083
M3	Watermelon (Tropicana)	Thermal	Water, sugar, watermelon, grapefruit, and apple juice concentrate, citric acid, natural flavors	1750	2.00	0.034
M4	MLN Watermelon (WTR)	Cold	Watermelon flesh and rind, organic lemon, ginger juice, organic ginger extract	354	2.98	0.248
M5	Sugar Kiss Juice Raspberry (Kiss Melon)	Cold	Melon, raspberry, lemon	354	3.99	0.333
M6	Summer Kiss Juice (Kiss Melon)	Cold	Melon, ginger, mint	354	3.99	0.333
M7	Honey Kiss Juice (Kiss Melon)	Cold	Melon, pear, lemon	354	3.99	0.333
M8	Sugar Kiss Juice Apple (Kiss Melon)	Cold	Melon, apple, lemon	354	3.99	0.333
M9	Melon Garden Greens Juice (Kiss Melon)	Cold	Melon, apple, spinach, kale, romaine lettuce, celery	354	3.99	0.333
M10	Melon Beet Boost Juice (Kiss Melon)	Cold	Melon, beet, ginger, mint, cucumber	354	3.99	0.333

* The above juices were categorized based on assumption that those juices may contain the major amount of respective juices (kale, beet, melon). However, labels do not state the composition of each juice.

Table A-3. Color attributes, brix, and pH of tested juices

Juice Type**	No.	Name of juice	L	a*	b*	C*	Hue	Brix	pH
Kale Juice	K1	Green Machine (Naked)	41.78	-5.33	13.21	14.24	-1.19	14.47	4.27
	K2	Healthy Greens (V8)	48.95	0.61	30.38	30.38	1.55	7.78	4.32
	K3	Grown Vegetable Juice (Estate)	38.28	9.64	13.27	16.40	0.94	6.00	4.41
	K4	Green Delight Organic (Suja)	44.59	-1.26	23.09	23.12	-1.52	13.17	4.00
	K5	Uber Greens Organic (Suja)	46.04	-3.39	21.72	21.99	-1.42	5.37	4.53
	K6	Radiant Probiotic Organic (Suja)	46.98	-6.81	25.17	26.08	-1.31	4.83	4.63
	K7	Organic Kale (1915)	48.95	-1.32	30.41	30.44	-1.53	9.55	3.91
	K8	Organic Emerald greens (Evolution)	41.49	-9.14	19.98	21.97	-1.14	6.50	4.65
	K9	Organic Green Devotion (Evolution)	45.23	-9.61	22.16	24.15	-1.16	4.85	4.65
	K10	Super Fruit Greens (Evolution)	35.92	-5.44	9.74	11.15	-1.06	13.23	4.39
Beet Juice	B1	Strawberry Banana (V8)	44.79	39.67	28.55	48.87	0.62	11.98	4.01
	B2	Original 100% Vegetables juice (V8)	37.88	24.95	16.80	30.08	0.59	6.53	4.36
	B3	Original 100% Vegetables Juice Low Sodium (V8)	39.13	25.77	19.27	32.17	0.64	6.47	4.52
	B4	Spicy Hot 100% Vegetables Juice (V8)	38.42	28.20	20.57	34.90	0.63	6.63	4.39
	B5	Beet Juice (Biotta)	24.64	15.99	0.36	16.00	0.02	12.15	4.74
	B6	Organic Pure Beet (Lakewood)	26.52	17.72	1.19	17.75	0.07	10.20	4.62
	B7	Farms Daily Roots (Bolthouse)	39.13	25.77	19.27	32.17	0.64	10.35	4.59
	B8	Very Veggie (R.W. Knudsen)	38.10	27.92	16.20	32.28	0.53	6.25	4.49
	B9	Organic Beet (1915)	24.92	4.74	-1.30	4.92	-0.27	9.13	4.46
	B10	Organic Cucum Berry (Simple Truth)	50.14	13.40	14.80	19.97	0.84	10.40	4.16
Melon Juice	M1	Synergy Watermelon Wonder (GTS)	59.41	20.78	20.06	28.88	0.77	6.13	3.47
	M2	Antioxidant Infusion (Bai)	73.95	28.42	-0.63	28.43	-0.02	3.20	3.25
	M3	Watermelon (Tropicana)	78.19	10.02	9.43	13.75	0.76	10.03	3.38
	M4	MLN Watermelon (WTR)	48.27	36.12	24.59	43.69	0.60	8.93	4.70
	M5	Sugar Kiss Juice Raspberry (Kiss Melon)	67.71	10.99	28.61	30.65	1.20	14.07	4.67
	M6	Summer Kiss Juice (Kiss Melon)	74.62	-4.89	26.08	26.53	-1.39	11.88	4.95
	M7	Honey Kiss Juice (Kiss Melon)	75.69	4.93	29.07	29.49	1.40	14.15	4.40
	M8	Sugar Kiss Juice Apple (Kiss Melon)	71.69	6.35	30.67	31.32	1.37	11.33	4.86
	M9	Melon Garden Greens Juice (Kiss Melon)	49.83	-4.36	29.74	30.06	-1.43	11.90	4.80
	M10	Melon Beet Boost Juice (Kiss Melon)	30.11	32.35	6.47	32.99	0.20	11.57	4.84

**Categorized based on assumption that those juices may contain the major amount of respective juices (kale, beet, melon). However, labels do not state the composition of each juice.

Table A-4. Solvents used for extraction of betalains.

Solvent Combination	Abbreviation
Water	S1
Water: ascorbic acid = (98:2)	S2
Water: formic acid = (98:2)	S3
Water: acetic acid = (98:2)	S4
Ethanol	S5
Ethanol: water = (70:30)	S6
Ethanol: water: ascorbic acid = (40:58:2)	S7
Ethanol: water = (30:70)	S8
Ethanol: water: ascorbic acid = (30:68:2)	S9
Ethanol: water: formic acid = (30:68:2)	S10
Ethanol: water: acetic acid = (30:68:2)	S11
Ethanol: water: formic acid = (18:80:2)	S12
Methanol	S13
Methanol: water = (70:30)	S14
Methanol: water: ascorbic acid = (40: 58: 2)	S15
Methanol: water = (30:70)	S16
Methanol: water: ascorbic acid = (30:68:2)	S17
Methanol: water: formic acid = (30:68:2)	S18
Methanol: water: acetic acid = (30:68:2)	S19
Methanol: water: formic acid = (18:80:2)	S20

Table A-5. Mineral contents in conventional and organic beets.

	Red beets		Golden beets	
Minerals	Conventional	Organic	Conventional	Organic
N	2.99 ± 0.12^{bc}	2.78 ± 0.01^{c}	3.82 ± 0.10^{a}	3.09 ± 0.10^{b}
P	2327.30 ± 56.25^{b}	2102.77 ± 11.85^{c}	3287.94 ± 83.62^{a}	3423.79 ± 211.35^{a}
K	27816.20 ± 771.65^{c}	32700.80 ± 648.88^{a}	30458.7 ± 388.57^{b}	28175.00 ± 1255.13^{c}
Ca	1597.72 ± 43.17^{a}	1716.42 ± 84.39^{a}	1648.74 ± 74.94a	795.19 ± 67.81^{b}
Mg	2448.46 ± 56.62^{a}	2096.09 ± 95.04^{b}	2085.55 ± 22.44^{b}	1492.88 ± 4.90^{c}
Na	12862.00 ± 449.32^{c}	9998.01 ± 96.41^{d}	20903.60 ± 720.57^{a}	18155.40 ± 162.36^{b}
Zn	26.02 ± 3.31^{a}	26.09 ± 1.60^{a}	25.21 ± 0.67^{a}	21.46 ± 0.87^{b}
Fe	55.07 ± 3.30^{a}	47.99 ± 2.98^{b}	60.57 ± 4.85^{a}	29.93 ± 2.86^{c}
Cu	7.00 ± 0.37^{c}	29.30 ± 4.59^{a}	14.48 ± 0.16^{b}	10.40 ± 0.31^{bc}
Mn	32.98 ± 0.63^{b}	28.74 ± 0.87^{c}	38.95 ± 0.61^{a}	22.68 ± 0.85^{d}
S	1235.59 ± 37.14^{b}	1537.87 ± 33.53^{a}	1450.92 ± 10.13^{a}	1202.89 ± 82.66^{b}
B	21.15 ± 0.51^{b}	18.24 ± 0.14^{c}	22.93 ± 0.48^{a}	16.82 ± 0.23^{d}

Different letters followed by values represented significant difference. Nitrogen was expressed as %, other minerals are mg/kg.

Table A-6. Levels of total betalains (betanin, isobetanin, valine-betaxanthin, and vulgaxanthin I) in extracts from conventional and organic beets.

		Red Beets		Golden Beets	
Solvent	Code	Conventional	Organic	Conventional	Organic
Water	S1	2990.1	524.5	347.6	0.0
Water: ascorbic acid = (98:2)	S2	1138.4	5254.6	495.8	289.4
Water: formic acid = (98:2)	S3	1969.8	3991.5	0.0	30.8
Water: acetic acid = (98:2)	S4	4036.9	3859.7	77.9	9.6
Ethanol	S5	1168.1	4307.4	253.1	158.6
Ethanol: water = (70:30)	S6	4038.9	5056.0	526.5	855.0
Ethanol: water: ascorbic acid = (40:58:2)	S7	1932.4	5760.3	394.5	395.9
Ethanol: water = (30:70)	S8	3517.3	5646.0	485.5	339.0
Ethanol: water: ascorbic acid = (30:68:2)	S9	2219.4	4002.8	522.0	588.8
Ethanol: water: formic acid = (30:68:2)	S10	3001.0	4069.0	72.6	68.9
Ethanol: water: acetic acid = (30:68:2)	S11	3678.7	2450.0	0.0	17.4
Ethanol: water: formic acid = (18:80:2)	S12	1567.3	4676.8	108.1	156.7
Methanol	S13	3833.8	12485.6	676.7	730.8
Methanol: water = (70:30)	S14	2073.8	4165.9	452.9	443.0
Methanol: water: ascorbic acid = (40: 58: 2)	S15	1555.5	7930.8	780.5	395.9
Methanol: water = (30:70)	S16	1904.6	5544.0	243.2	617.5
Methanol: water: ascorbic acid = (30:68:2)	S17	2816.3	3223.6	816.4	755.8
Methanol: water: formic acid = (30:68:2)	S18	3409.6	4876.6	140.6	92.8
Methanol: water: acetic acid = (30:68:2)	S19	2967.9	6418.4	0.0	35.7
Methanol: water: formic acid = (18:80:2)	S20	2174.8	11923.9	0.0	1316.2

Values were expressed as µg/g.

APPENDIX B

FIGURES FOR ADDITIONAL INFORMATION

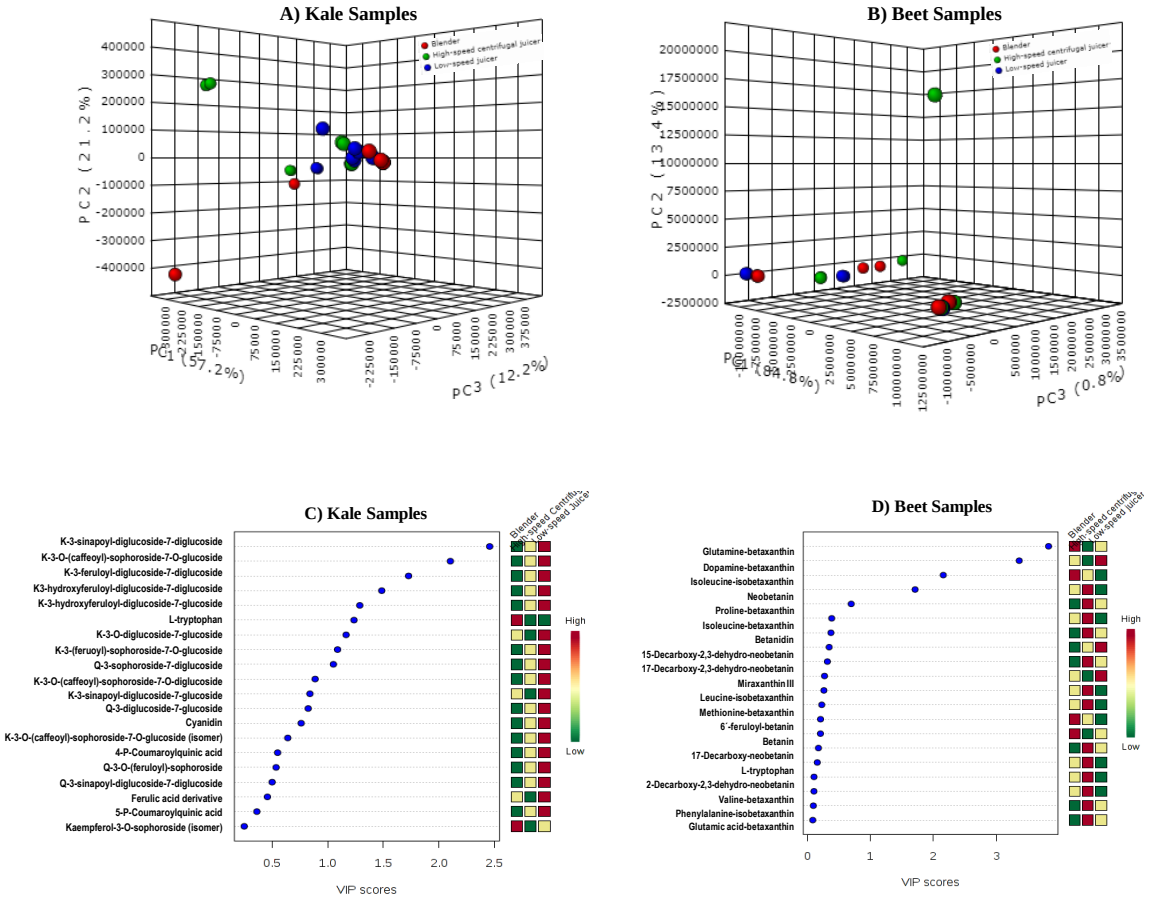

Figure B-1. Multivariate data analysis revealed the influence of processing techniques on metabolites. Principal component analysis (PCA) of (A) kale juices and (B) juices. Variable importance of projection (VIP) scores presents a overview of abundance of metabolites, with the degree of color saturation indicates the level of metabolites.

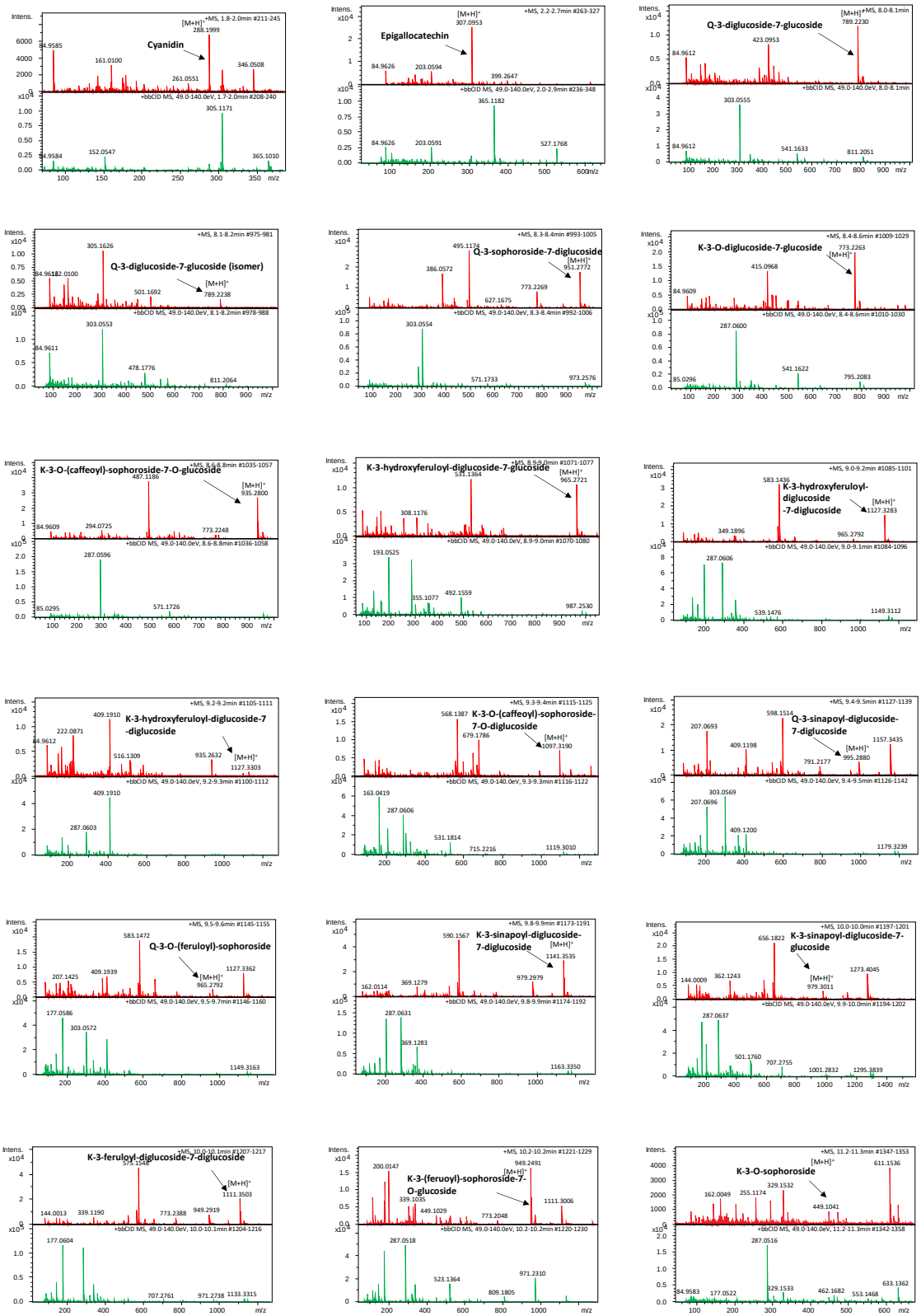

Figure B-2. Protonated mass spectra of phytochemical compounds of retention time 1-11min in extracts of *Brassica* vegetables.

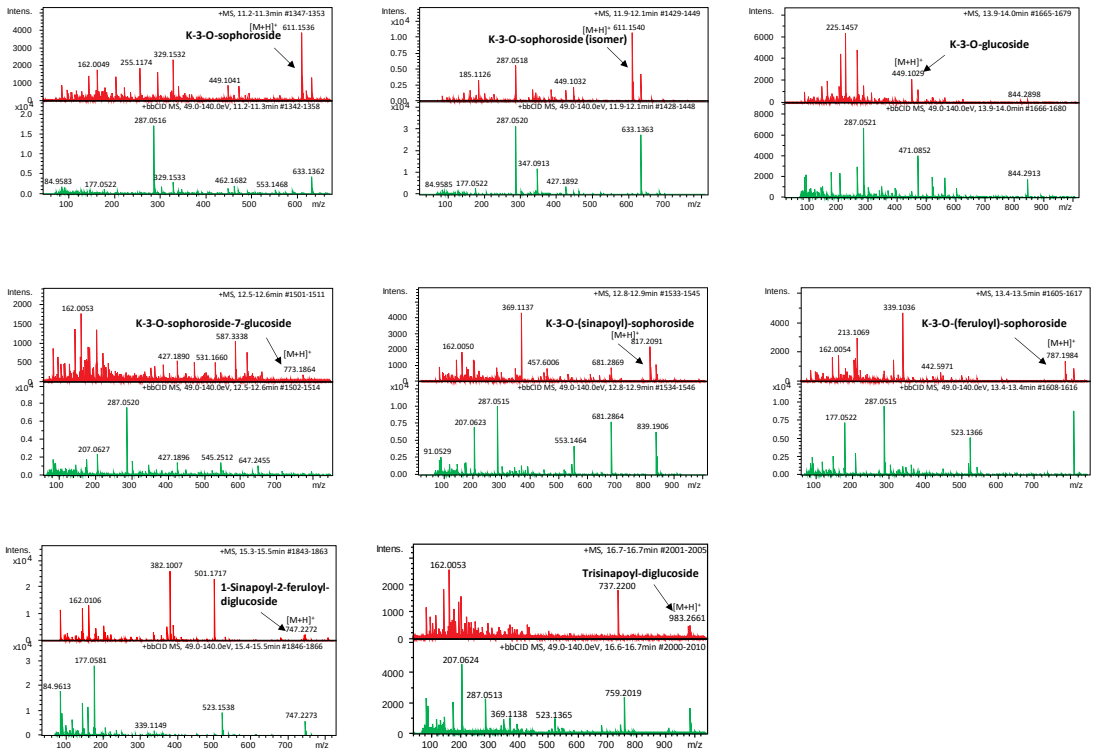

Figure B-3. Protonated mass spectra of phytochemical compounds of retention time 11-16min in extracts of *Brassica* vegetables.

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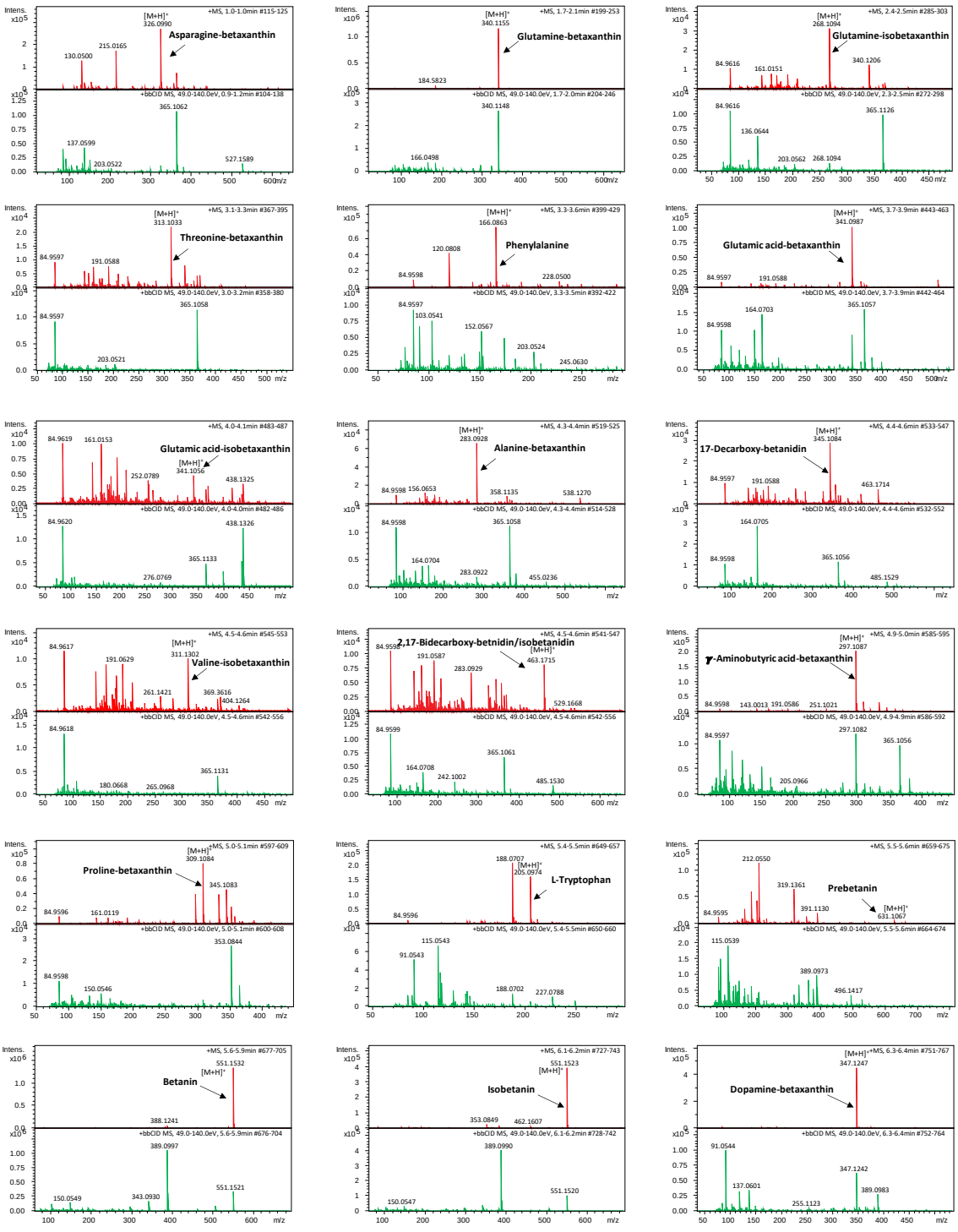

Figure B-4. Protonated mass spectra of phytochemical compounds of retention time 1-6min in beet extracts.

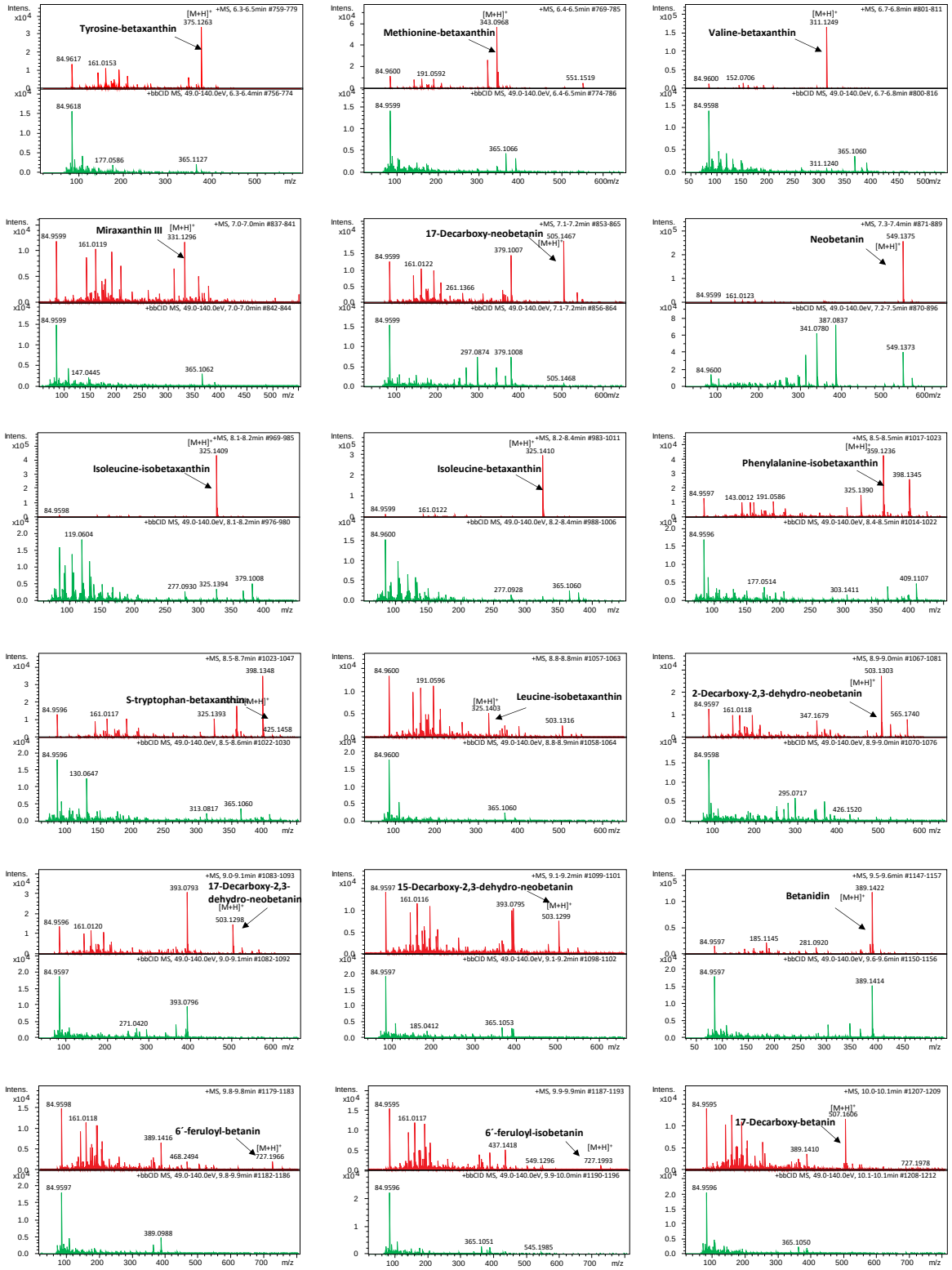

Figure B-5. Protonated mass spectra of phytochemical compounds of retention time 6-10min in beet extracts.

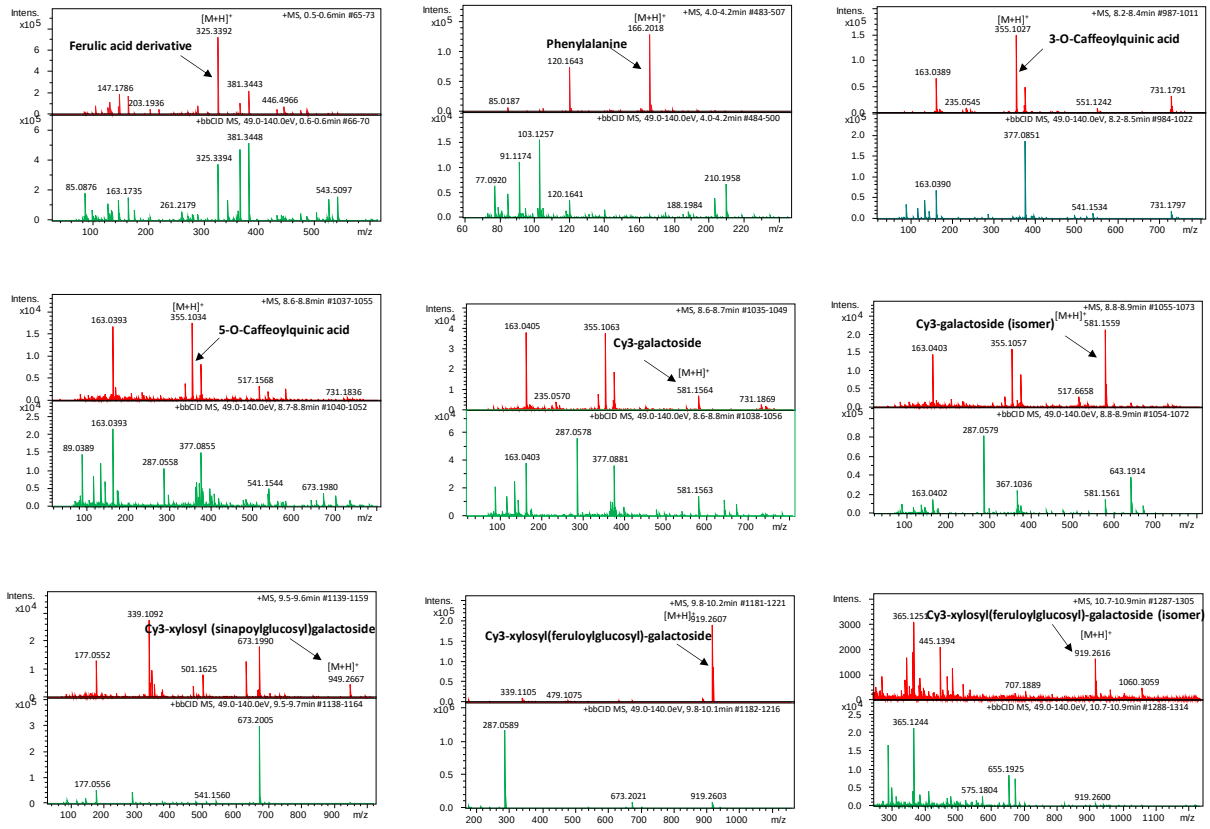

Figure B-6. Protonated mass spectra of phytochemical compounds of retention time 1-10min in carrot extracts.

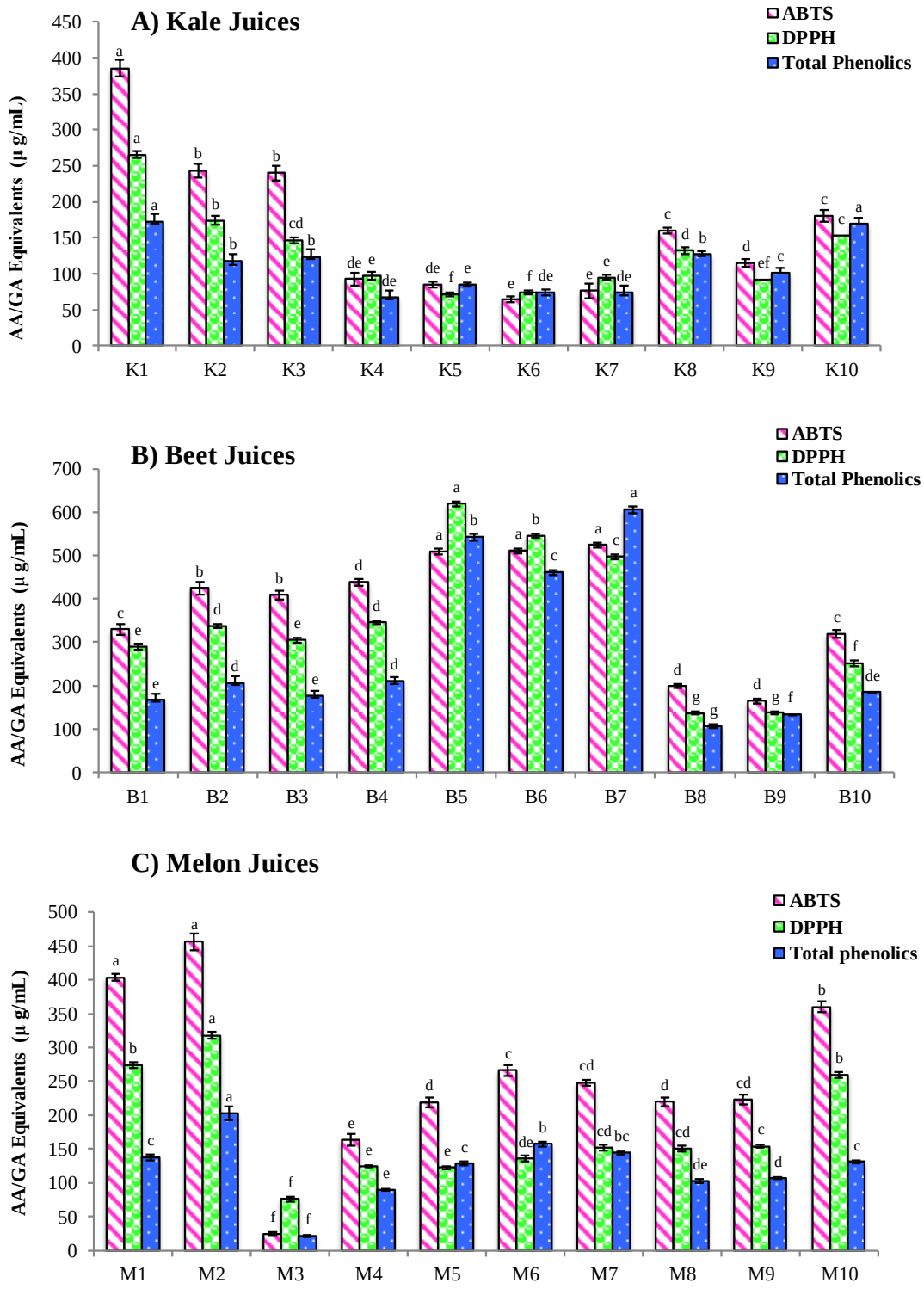

Figure B-7. Free radical scavenging activities and total phenolics of commercial juices: (A) kale juices; (B) beet juices; (C) melon juices. DPPH and ABTS were expressed as ascorbic acid (AA) equivalents, and total phenolics were expressed as gallic acid (GA) equivalents. Values followed by the same letter in the same assay are not significantly different ($p<0.05$).

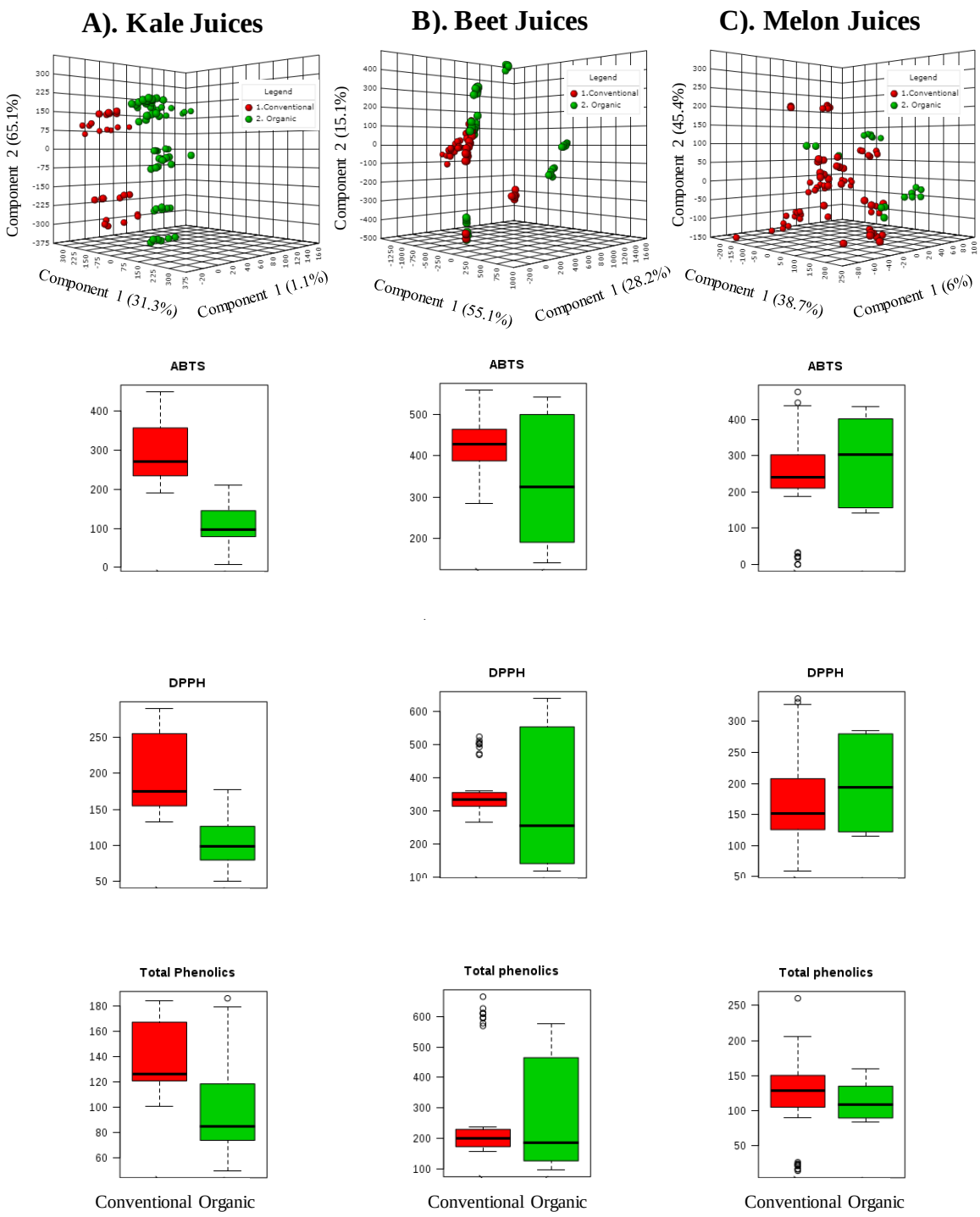

Figure B-8. PLS-DA and antioxidant activities of different juices. (A) Kale juices; (B) Beet juices; (C) Melon juices. The red clustered dots represent juices obtained from conventional raw materials, and the green grouped dots were juices partially obtained from organic raw materials. DPPH and ABTS were expressed as ascorbic acid (AA) equivalents, and total phenolics were expressed as gallic acid (GA) equivalents.

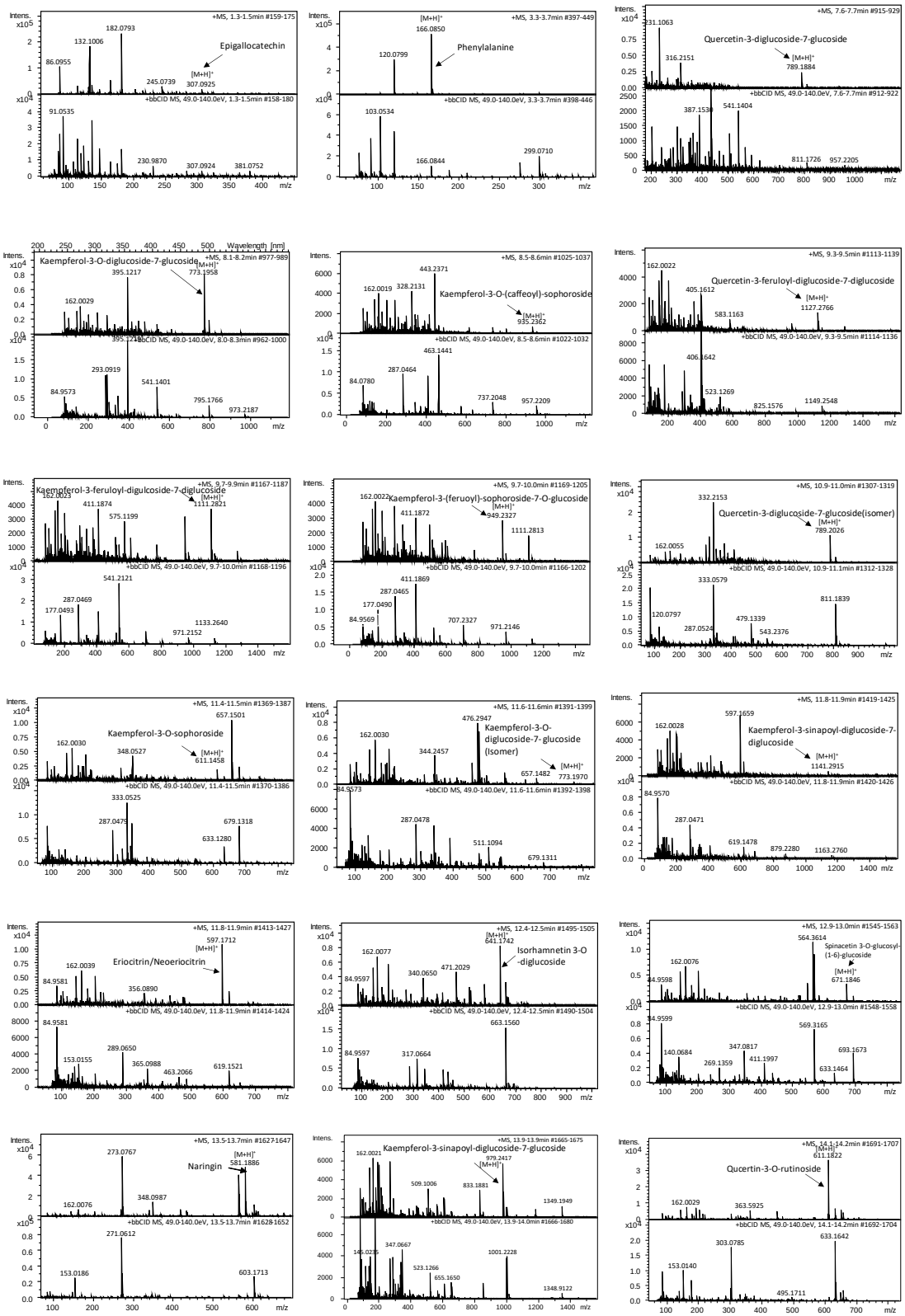

Figure B-9. LC-MS spectra of identified compounds in kale juice samples.

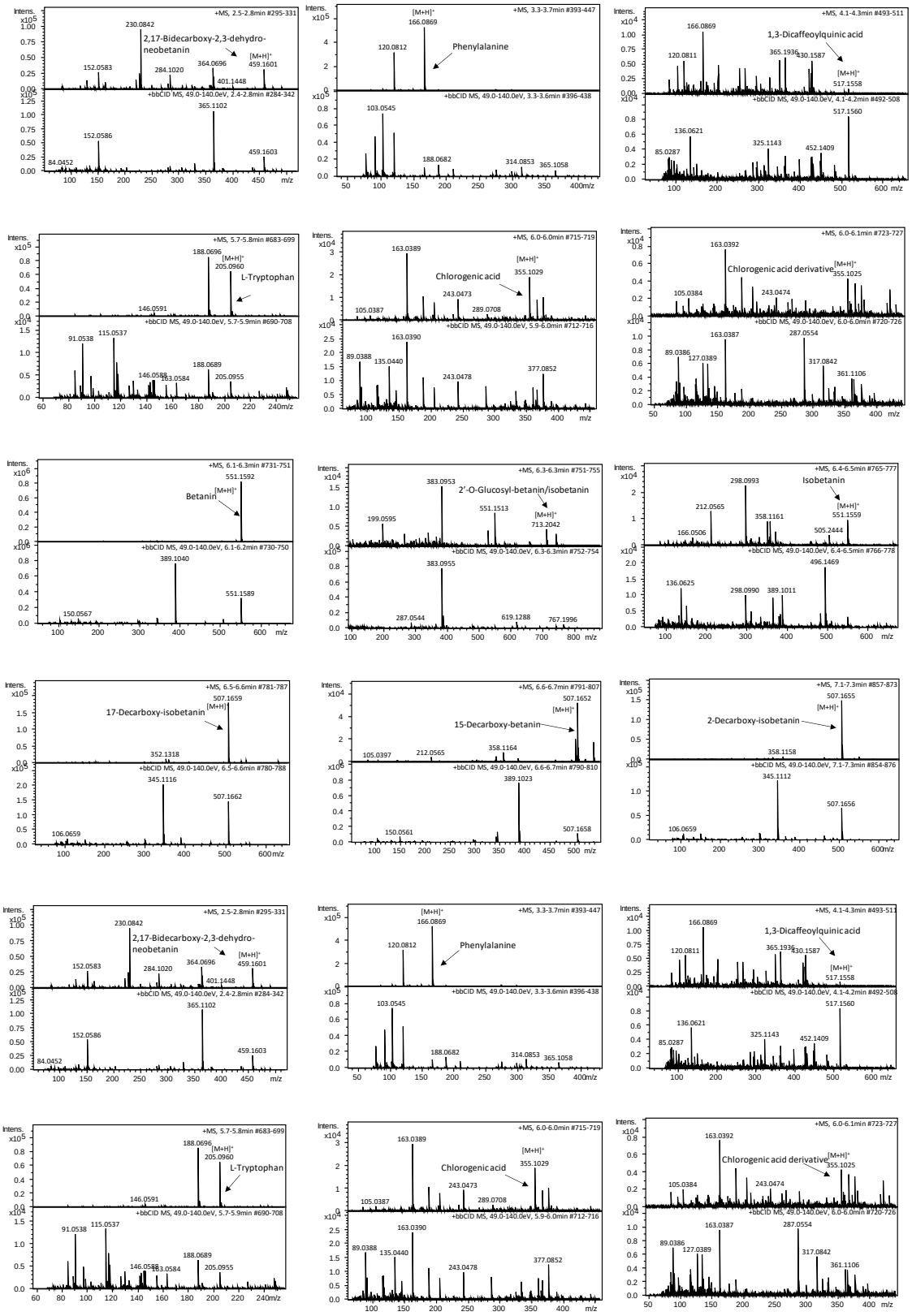

Figure B-10. LC-MS spectra of identified compounds in beet juice samples.

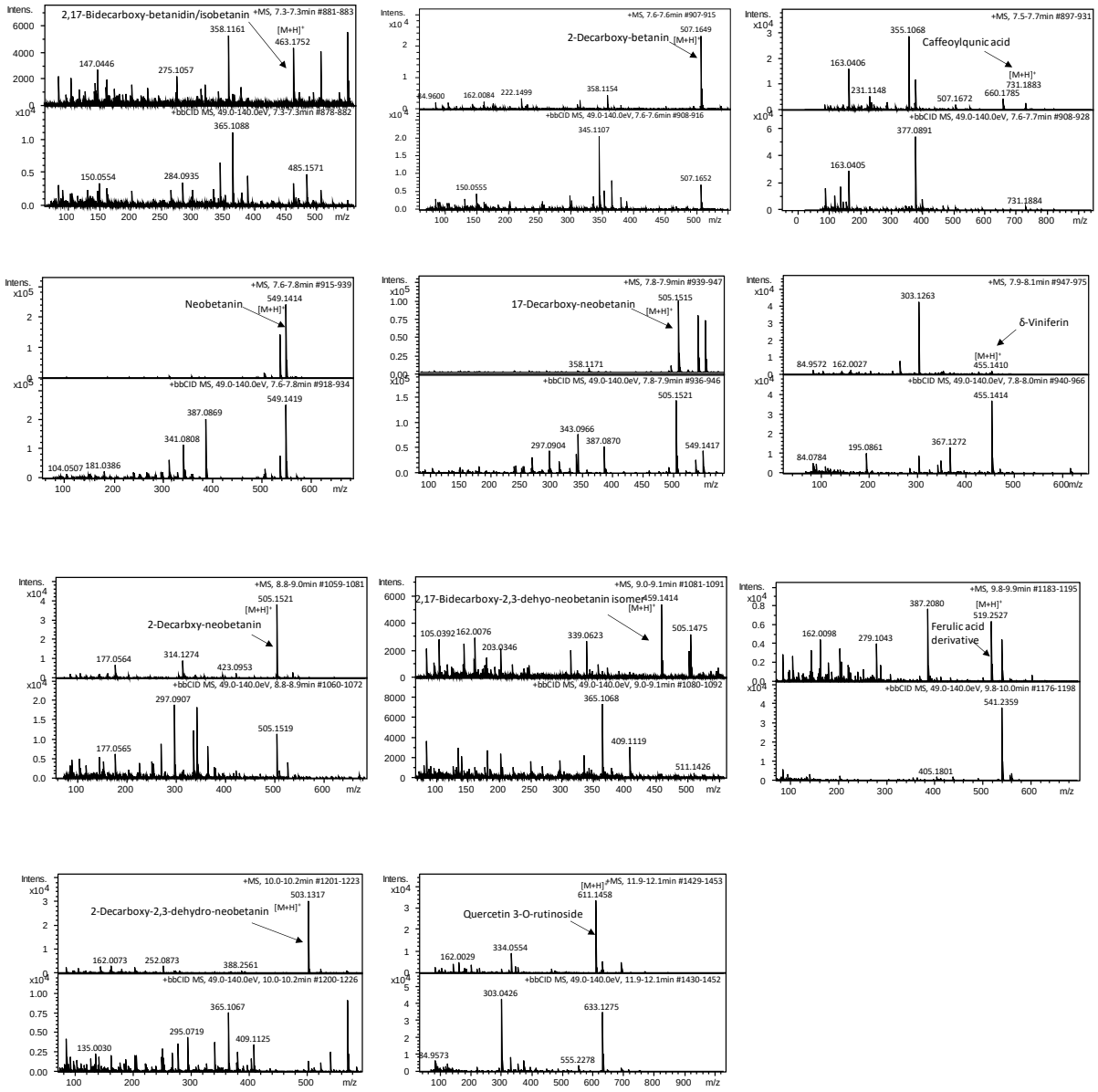

Figure B-10. Continued

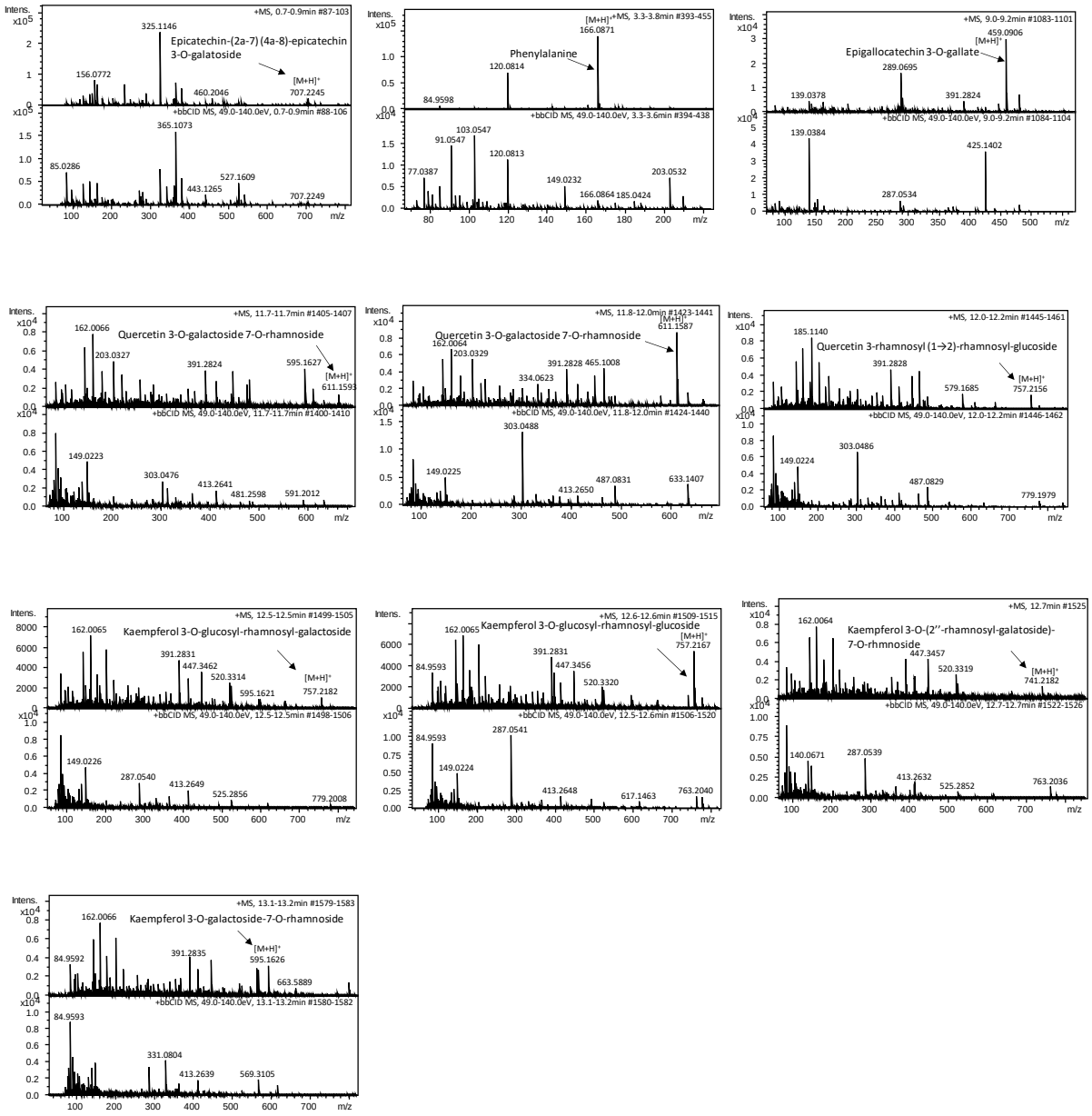

Figure B-11. LC-MS spectra of identified compounds in melon juice samples.

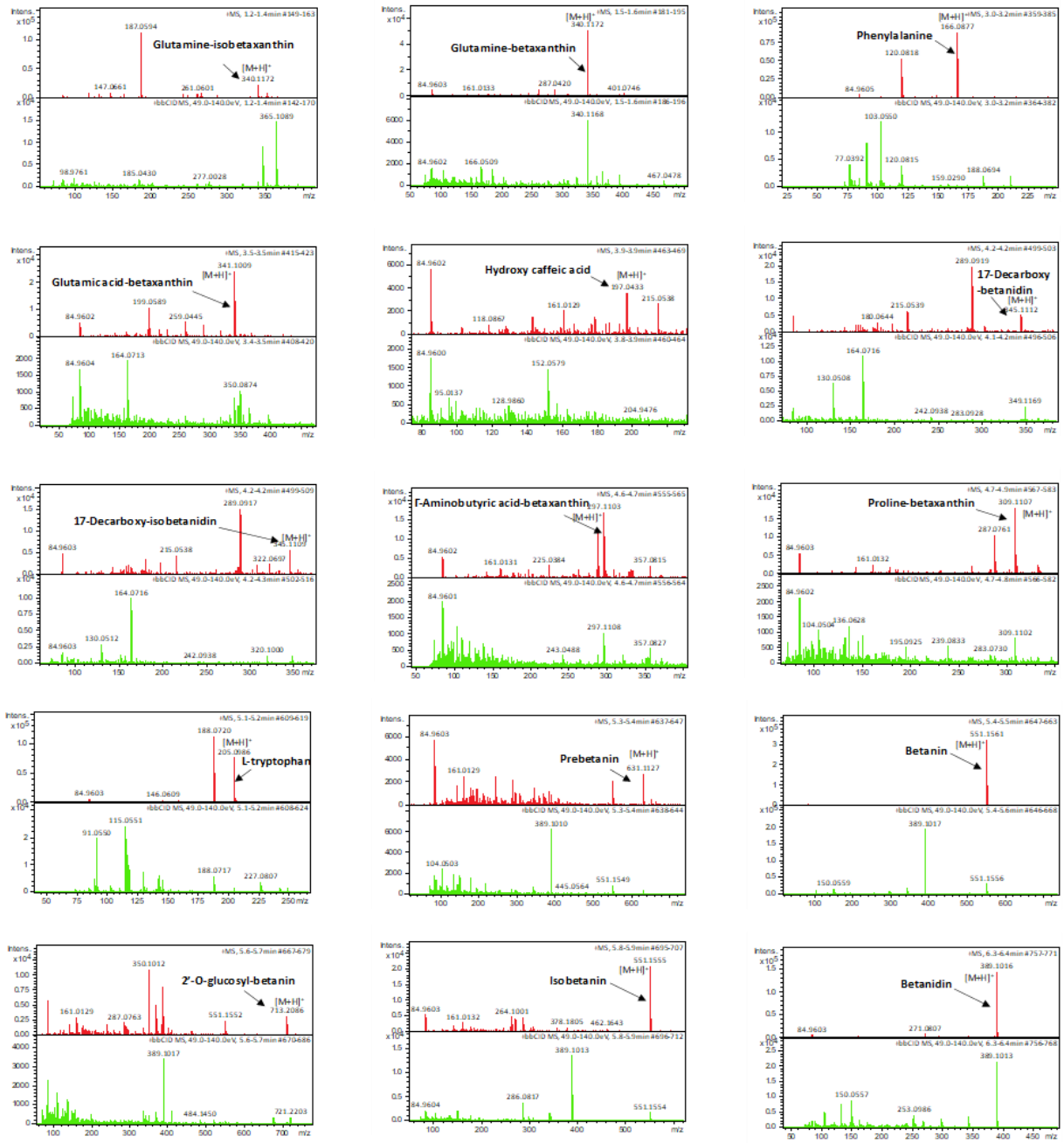

Figure B-12. Identified betalains with retention times 1.2 to 7.0 min, from conventional and organic red and golden beet samples.

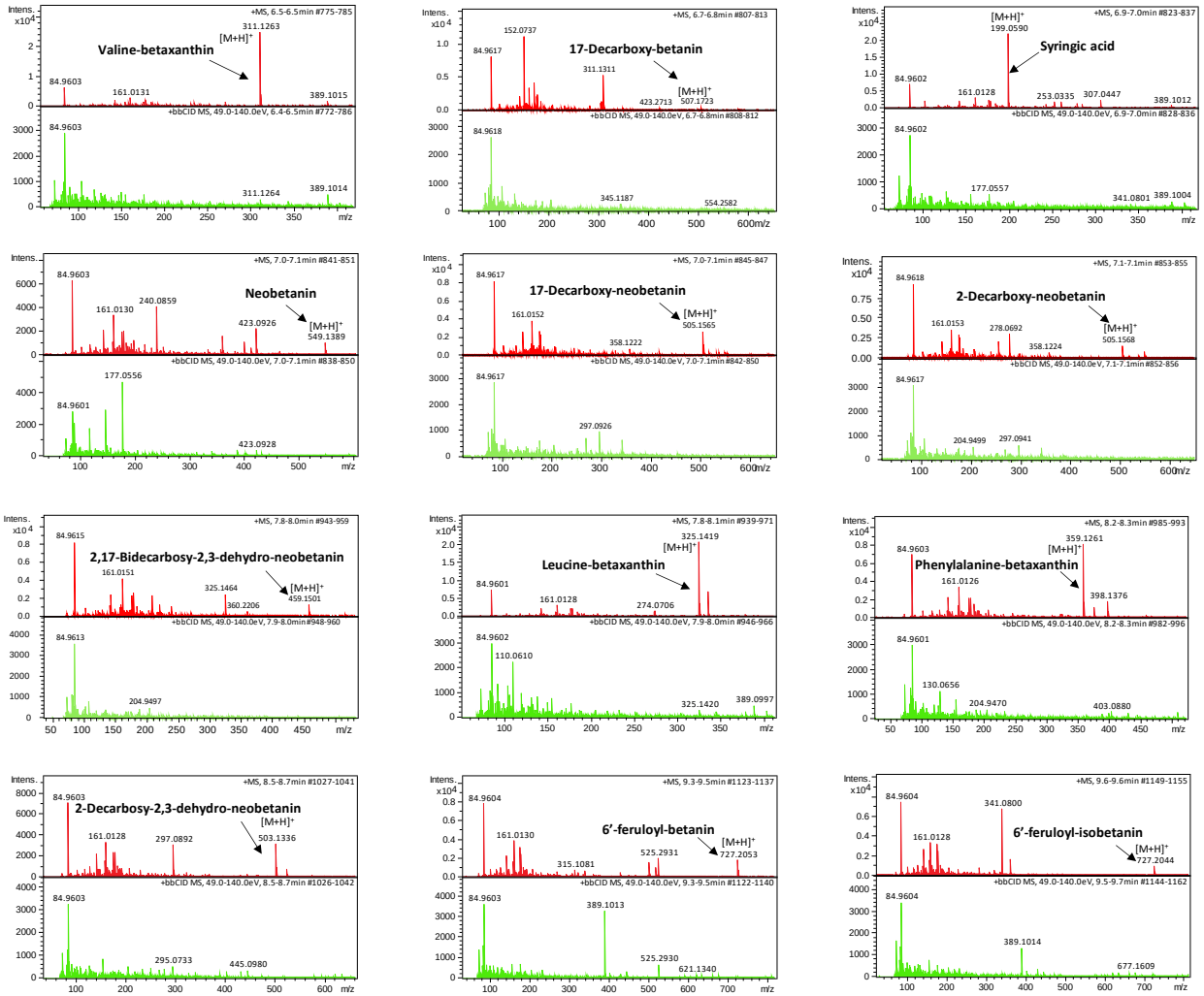

Figure B-13. Identified bioactive compounds with retention times from 7.0 to 9.6 min, from conventional and organic red and golden beet samples.