INNOVATIVE COLD PLASMA-ASSISTED EXTRACTION FOR BIOACTIVE COMPOUNDS FROM AGRICULTURAL BYPRODUCTS

by

Yiwen Bao

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Food Science

West Lafayette, Indiana

May 2020

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Jen-Yi Huang, Chair
Department of Food Science

Dr. Lavanya Reddivari
Department of Food Science

Dr. Alexey Shashurin
School of Aeronautics and Astronautics

Approved by:
Dr. Arun Bhunia

Dedicated to my family

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Jen-Yi Huang, for his generous support and mindful guidance on my master's projects. Whenever I had questions or problems that I couldn't solve by myself, he always came up with timely solution or suggestions that worked around. His patient helps as well as strict adamic attitude encouraged me to overcome difficulties during researches and eventually completed this thesis. I would also like to thank my committee members, Dr. Reddivari and Dr. Shashurin, for their constructive suggestions and helps.

Many thanks to people who provide their kind helps to my projects. I would like to thank Red Gold company and Dr. Yanyun Zhao from Oregon State University for providing fruit pomace materials. For the analysis part, I am grateful to Dr. Qin Xu, Dr. Stephen Lindemann, Dr. Allen Garner, Dr. Owen Jones and Dr. Jozef Kokini for their approvals to access the equipment and to their students for instructing me to use the equipment.

I am also grateful to my colleagues and friends at Purdue for their helps and encouragements during my master's study. Finally, I would like to thank my family for their supports and loves. Thank you for being there every step of the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

HVACP	High voltage atmospheric cold plasma
PC	Phenolic compounds
TP	Tomato pomace
GP	Grape pomace
TPC	Total phenolic content
TAC	Total anthocyanin content
AA	Antioxidant activity
OES	Optical emission spectroscopy
SEM	Scanning electron microscopy
UPLC	Ultra-performance liquid chromatography

ABSTRACT

Fruits play a necessary role in the human diet, and their cultivation is important to the prosperity of any country worldwide. However, fruit waste generated in large quantities in agricultural value chain is normally used to feed animals or directly disposed to landfill, ending up with low economic value and a heavy environmental burden. Agricultural waste that contains significant amounts of bioactive compounds can be utilized as byproducts and valorized through bioactives recovery. Conventional bioactive compounds extraction includes intensive uses of organic solvents and also has relatively low efficiency. Therefore, an environment-friendly alternative with higher extraction efficiency is needed. Cold plasma can convert gaseous medium to a highly reacting state with low energy cost, generating reactive species that are able to disrupt cell structures as well as modify material surfaces. This study has developed an innovative cold plasma-assisted extraction technology to enhance the recovery of bioactive compounds from fruit processing byproducts. The objectives of this study are to examine the effects of dielectric barrier discharge plasma on fruit pomaces, in terms of (i) surface microstructure and properties, (ii) extraction efficiency of their bioactive compounds, and (iii) bioactives composition and nutritional value of their extracts.

High voltage atmospheric cold plasmas (HVACP) generated with different working gases (air, argon, helium and nitrogen) were applied on tomato pomace (TP). In addition to creating ruptures on TP epidermal cells, HVACP treatments were found to decrease the water contact angles of tomato peels and accelerate the drying of tomato fruits, indicating the formation of more hydrophilic surfaces. Helium and nitrogen plasmas-treated TP showed increased PC extraction yields by 10%, and all HVACP-treated samples exhibited higher AA and changes in their phenolic compositions.

Grape pomace (GP) from red wine production was treated by helium-HVACP for different time periods (5, 10 and 15 min). Similar cell structure disruption and surface hydrophilicity enhancement were observed, and the effects became more significant as treatment extended. HVACP treatment also increased the total phenolic content in GP extracts, by 10.9−22.8%, which contained a higher anthocyanin concentration and showed an improved AA (16.7−34.7%). Furthermore, competitive effects of HVACP treatment on PC extractability enhancement and their degradation were observed.

The results of this study have proved that HVACP-assisted extraction successfully improved the extraction efficiency of bioactive compounds from fruit pomaces and enhanced the nutritional quality of their extracts. This novel technology is a promising method for valorizing different agriculture byproducts into functional food ingredients and nutraceuticals with high nutritional values, which thus can bring significant economic benefits to the agricultural, food and nutraceutical industries.

CHAPTER 1. INTRODUCTION

1.1 Fruit processing

Fruits play an important role in human diet. Along with macro-nutrients (carbohydrate, protein, lipids), fruits consist of significant amounts of multiple micro-nutrients, such as vitamins, polyphenols, etc., that are highly essential for maintaining human health. All over the world, the most popular fruits include apple, orange, grape as well as banana. The U.S. is one of the main fruit producers with an estimated average annual production of 28 million tons and makes over $25 billion for national agricultural economy (USDA ERS, n.d.). Although the majority of fruits can be eaten in raw form, large production guarantees enough raw fruits for consumption and also for processing fruits at different levels into various fruit products is a common practice in the food industry.

Minimal (basic) fruit processing such as washing, cutting, blanching, pasteurization, addition of food preservatives for disinfestation and sanitization (Jideani et al., 2017), as shown in Figure 1.1, aim to extend the shelf life of fruits and make them ready-to-eat. On the other hand, further processing including pressing, heat treatments, fermentation and drying can change the inherent attributes of fruits and convert them to other value-added products, such as tomato paste and ketchup, wine, fruit juices and dried fruits.

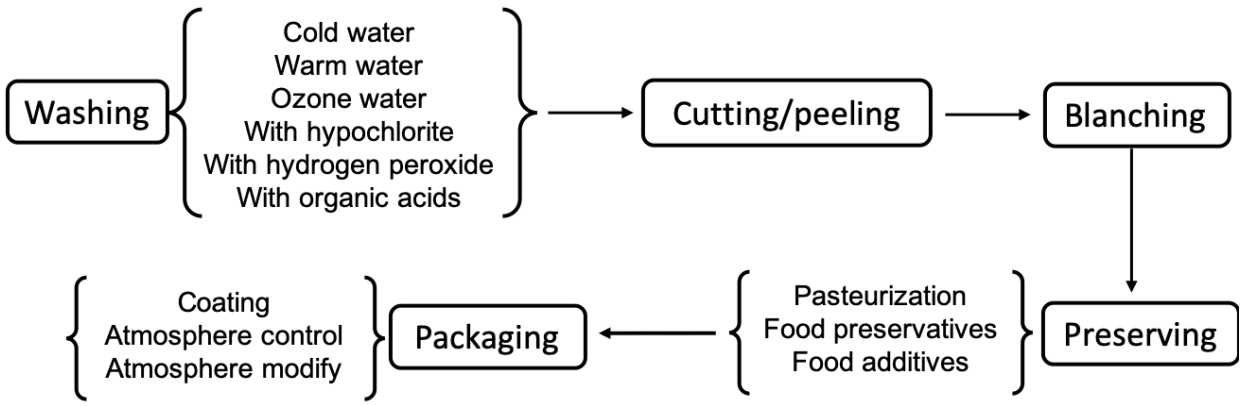

Figure 1.1 Flow diagram of minimal fruit processing.

Tomato (*Solanum lycopersicum*) is a perennial herbaceous plant which is grown in open field in tropical and temperate regions as well as in greenhouse under controlled temperature. Tomato is a climacteric fruit which can continue to ripen after harvested due to increasing ethylene production. Commercially tomatoes have various sizes, shapes and colors depending on their breeding and cultivars. Tomato fruit is composed of a thin outer layer of peel, a fleshy tissue enclosing 50 to 200 seeds inside the locular cavities (OECD, 2016). Tomato comprises large amounts of water and carbohydrates and trace of proteins. Tomato is also a good source of micro-nutrients (vitamins and minerals) and other beneficial nutrients like antioxidants.

The production and consumption of tomatoes have increased rapidly because it can provide essential nutrients, including lycopene, vitamin C, beta-carotene and phenolic compounds, which are beneficial to human health (Gerszberg et al., 2015). It has an annual production over 14 million tons in U.S., while almost 86% are processed to different products (Kelley et al., 2010). Figure 1.2 shows the typical tomato puree and paste processing. Tomato peels and seeds are normally excluded during paste and puree processing. The operation of skin/seeds removal is composed of two steps. Firstly, tomatoes are peeled using either chemical or mechanical method. Chemical peeling is also known as lye-peeling that normally uses NaOH or KOH to remove

tomato skin, and mechanical peeling includes steam or hot water treatment (Garcia & Barrett, 2005). The second step is to remove seeds and peels using pulper/finisher and collect in a separate container as tomato pomace. Commercial tomato processing can generate 5−10% tomato pomace (mainly peels and seeds), which has caused a severe waste problem (Ventura et al., 2009).

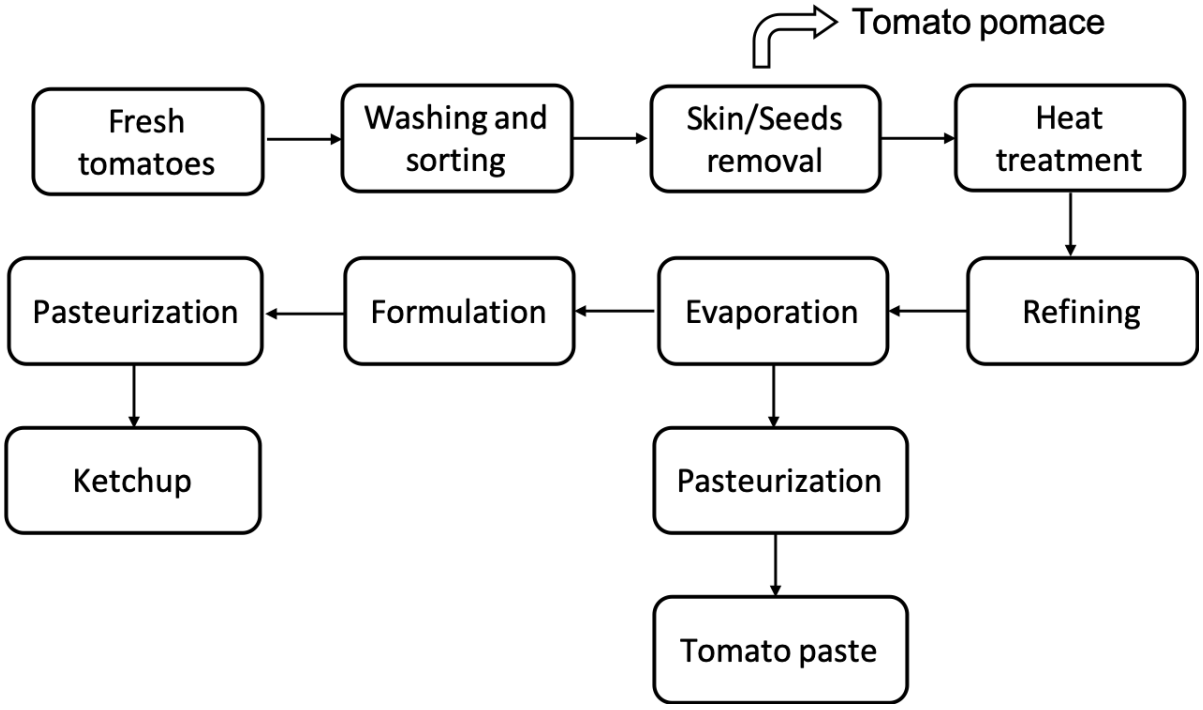

Figure 1.2 Tomato ketchup and paste processing flow diagram. Adapted from Gould (1992).

Grape (*Vitis* sp.) is a non-climacteric fruit that grows in clusters and composes of skin, fruit tissue and seeds. Grape has many species varying with geographical locations which are grown for different products and can be divided into wine grapes, table grapes and raisin grapes. Among these species, wine grapes (*Vitis vinifera*) are the most widely grown cultivars due to the large demand of winemaking (Reisch, Owens & Cousins, 2011).

Grape is also one of the most popular crops in the U.S., with over 7.5 million tons produced every year. Almost 88% of harvested grapes are processed to different products (USDA, 2019).

Grape processing usually generates 20% grape pomace (composed of stems, skins and seeds). For example, grape juice production has a low process yield of around 72 % (w/w) (Lieu & Le, 2010), and the rest of the fruit biomass becomes waste. Similarly, a large amount of waste is generated in grape wine production. Figure 1.3 presents the processes in red wine production. After fermentation, must is pressed to release juice and separate skin, seeds and other solid parts which are known as grape pomace (Iannone et al., 2016). Grape pomace is generally used with low economic values, for example, for animal feeding, or directly disposed to landfill (Arvanitoyannis & Varzakas, 2008).

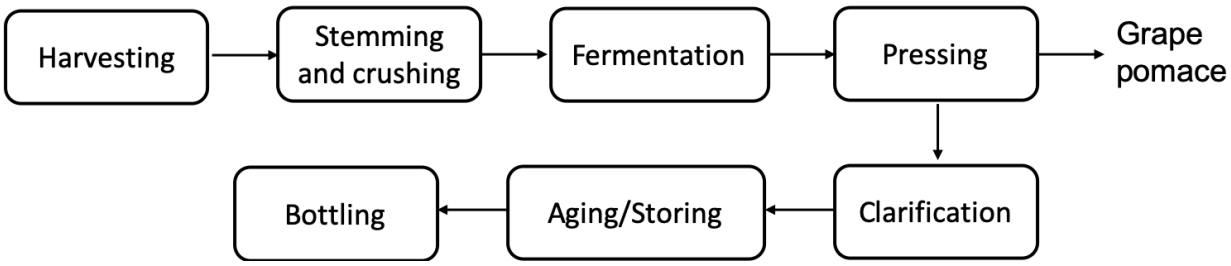

Figure 1.3 Flow diagram of red wine production. Adapted from Lasik et al. (2018).

The waste generated from fruit processing still has high contents of natural micro-nutrients and bioactive compounds, like phenolics. For example, Valdez-Morales et al. (2014) reported the content of phenolic compounds in tomato pomace of 138.9 mg gallic acid equivalents (GAE)/100 g. For wine grape pomace, the value was estimated to be 970−2650 mg GAE/100 g dry weight (Spigno et al., 2007). With such high phenolic content, tomato and grape pomaces show great potential for utilization as processing byproducts, instead of waste, for conversion to value-added functional food ingredients and nutraceuticals.

1.2 Phenolic compounds

Plants have both primary and secondary metabolisms. The primary metabolism produces substances that are essential to cell maintenance including carbohydrates, lipids and proteins, while secondary metabolites are formed through subsidiary pathways. Phenolic compounds (PC), as the secondary metabolites, are widely present in plants, and different plants have distinct phenolic profiles. Table 1.1 summarizes the most common PC found in fruits, plants and their products. PC are typically generated from two pathways: phenylpropanoid pathway and acetic acid pathway. Most PC are synthesized from the former and flavonoids are formed by the combination of both (Giada, 2013). PC ranges from simple phenolic molecules to highly polymerized compounds, but have a basic chemical structure composed by at least one aromatic ring with one or more hydroxy groups attached (Balasundram, Sundram, and Samman, 2006). Most of the PC in plants are linked with different sugar units, and acylated sugars can also attach to different positions of the phenolic skeletons (Tsao, 2010). According to the chemical structure of aglycones, PC can be classified into five main categories: phenolic acids, flavonoids, stilbenes, coumarins and tannins, in which phenolic acids and flavonoids can be further divided into several subgroups, as shown in Figure 1.4.

Table 1.1 Common phenolic compounds in food (Aires, 2017).

	Compound	Common food source
Phenolic acids		
	Hydroxycinnamic acids	Cereals, coffee, cherries, citrus fruits and juices, peaches, plums, spinach, tomatoes, wheat flour, corn flour, rice flour, potato, olive mill wastewaters, winery sludge from red grapes, artichoke wastewaters, almonds
	Hydroxybenzoic acids	Oilseeds, cereals, coffee, cowpeas, wheat flour, black currant, blackberry, raspberry, squash seeds and shell
Flavonoids		
	Anthocyanins	Grapes, red wine, grape seeds, grape skins, winery by-products, fermented grape pomace, strawberries, back and red currants, raspberries, plums, red cabbage
	Chalcones	Apples and apple juices,
	Flavanols	Apples, grapes, leeks, tomatoes, curly kale, onions, lettuces, berries, beans, red grapes, black and green tea, red wine and red winery by-products, cider
	Flavanones	Citrus fruits, citrus juices, orange peels and seeds wastes
	Flavonols	Apples, apple peels, beans, leeks, lettuce, onions, tomatoes, olive leafs, broccoli inflorescences, chestnut, olives and olive fermented pomaces
	Flavones	Spinach, citrus fruits, celery, pepper, capsicum pepper,
	Isoflavones	Soybeans, soy flour, soymilk, soy processing waste
	Stilbenes	Red grapes, grapes skins, grape seeds, red grape fermented pomaces
	Xanthones	Mango fruits and mango peels fermented pomaces
Tannins		
	Condensed tannins	Apples, grapes, peaches, pears, chestnut, hazelnuts, nuts
	Hydrolyzable tannins	Pomegranate, raspberries

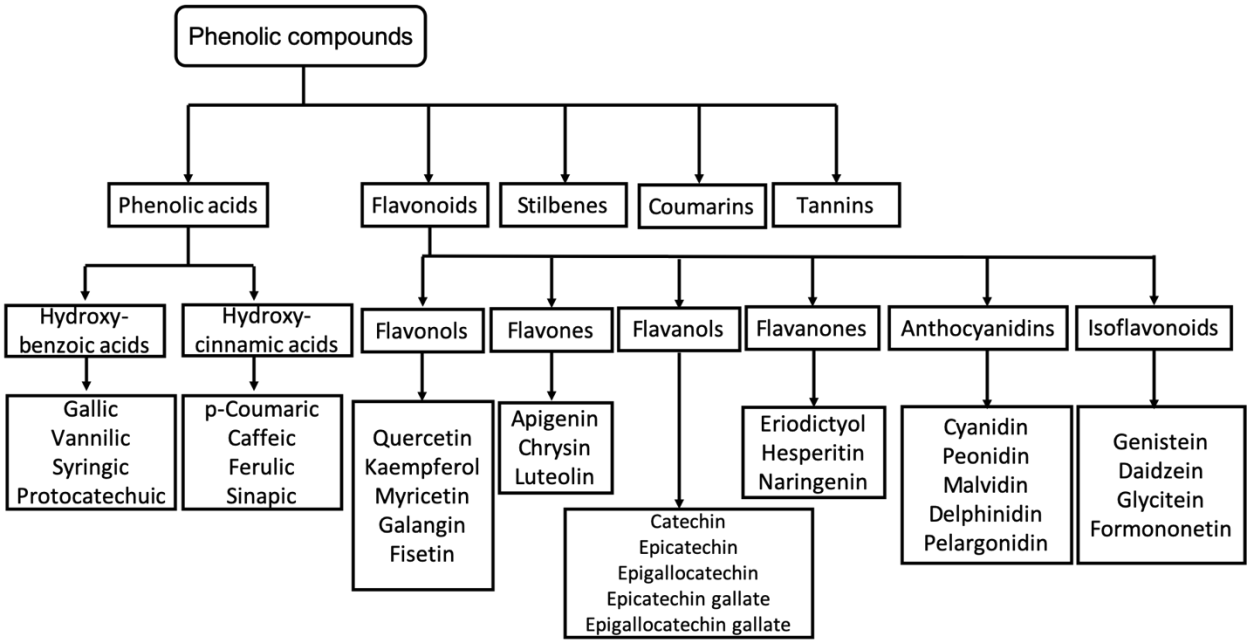

Figure 1.4 Classification of dietary phenolic compounds. Adapted from Bellik et al. (2013).

PC also have high economic values on the global phytonutrients market, which was valued at $3.05 billion in 2014 and is expected to reach $4.63 billion in 2020 (Markets and markets, 2015); Additionally, PC-based products play an important role in the nutraceutical market of an estimated value of more than $10 billion between 2013 and 2018 (Yahia, 2017). It is important to point out that anthocyanin, a specific type of PC, is an important natural colorant on the $305-million colorant market estimated in 2018 (Market Research Report, 2019).

In plant tissue, PC can be in either free form or bound form. Free PC are present in plant vacuole and cytoplasm and are organic solvent extractable. In contrast, extracting bound PC is more difficult because they are covalently bound to the polysaccharides of plant cell wall which can only be cleaved with additional energy applied. The plant cell wall structure not only hinders the extraction of bound PC, but also may even entrap free PC. Therefore, the choice of extraction method can greatly influence the PC extraction yield (Khoddami, Wilkes and Roberts, 2013).

Phenolic compounds extraction

Solid-liquid (SLE) and liquid-liquid extraction (LLE) are the most commonly used methods for PC recovery. SLE allows soluble components to be separated from solids using liquid solvent. The solvent should have a high affinity to the target components in solids. LLE is a method to separate compounds from liquid mixture, based on their relative solubility in two different immiscible liquids (polar vs. non-polar). The underlying mechanism of LLE is to create a lower free energy state among solvent and liquid mixture that makes the solutes and solvent in a stable configuration. The solvent that is enriched with solutes is called extract. Organic solvents like methanol, ethanol, acetone and their combinations with different proportions of water are widely used for SLE and LLE (Babbar et al., 2014). The choice of extraction solvent varies depending on the PC solubility of the sample. Table 1.2 summarizes the SLE/LLE methods reported in literature for different samples and their extraction conditions and yields.

Table 1.2 Conventional extraction method of PC for different plants.

Sample	Extraction solvent and condition	TPC (mg/100 g)	Reference
Seeds of *Sterculia apetala*	Water, 25 ºC, 6 h	257[a]	Mosca et al., 2018
	Water, 60 ºC, 6 h	413[a]	
	50% ethanol, 25 ºC, 6 h	237[a]	
	50% ethanol, 60 ºC, 6 h	472[a]	
Blackberry	Methanol + 1.2 M HCl, 95 ºC, 2 h	417−555[b]	Sellappan et al., 2002
Blackberry (Rabbiteye)	Methanol + 1.2 M HCl, 95 ºC, 2 h	270−930[b]	
Blackberry (Southern highbush)	Methanol + 1.2 M HCl, 95 ºC, 2 h	261−585[b]	
Tomato	80% methanol + 1% HCl, room temperature, 2 h	25.9−49.8[a]	Martínez-Valverde et al., 2002
	80% methanol, room temperature, 1 h	0.37−5.4[b]	Barros et al., 2012
Onion	50% methanol + 1.2 M HCl, 80 ºC, 2 h	84.5−316[b]	Nuutila et al., 2003
Garlic	50% methanol + 1.2 M HCl, 80 ºC, 2 h	9.5−29.5[b]	

[a] mg gallic acid equivalent (GAE)/100 g

[b] mg compound/100 g

However, there are two main concerns with these processes: extensive solvent use and relatively low extraction efficiency. For example, as one of the most commonly used solvents, the disadvantage of extracting with methanol is not only the potential solvent residues in extracted products which hinder their food/nutraceutical applications, but also the hazards to operating personnel and environment. Additional purification steps thus need to be conducted to ensure product safety as well as human and environmental health, which can consume more energy and cost (Brglez Mojzer et al., 2016). Besides, conventional solvent extraction methods are usually carried out at elevated temperatures (up to 50 ºC) for long processing time (up to 24 h), which are very energy-intensive and inefficient (Brglez Mojzer et al., 2016; Mokhtarpour et al., 2014). Furthermore, conventional PC extraction methods are not effective in extracting bound PC from plant matrix (Su et al., 2014). In order to recover bound PC from plant, alkaline or acid hydrolysis is normally used (Cheng et al., 2014; Madhujith & Shahidi, 2009). Hydrolysis also requires the use of strong acid or alkaline and needs to be operated at extremely high temperatures (80−95 ºC) for acid hydrolysis and 45 ºC for basic hydrolysis (Garcia-Salas et al., 2010), which is not environment-friendly and energy-efficient either.

In addition to conventional extraction, some other methods have been developed in order to enhance the extraction efficiency, such as Soxhlet extraction (Nn, 2015), microwave-assisted extraction (MAE) (Krishnaswamy et al., 2013), ultrasound-assisted extraction (UAE) (Espada-Bellido et al., 2017), and pressurized liquid extraction (PLE) (Solana et al., 2015). Table 1.3 summarizes the application of those alternative approaches. However, these methods also have some limitations. Although Soxhlet extraction consumes less solvent, due to the use of highly vaporized organic solvent, it causes potential hazards associated with exposure to toxic and flammable organic vapors and also produces negative environmental impacts (Nn, 2015).

Microwave-assisted extraction needs a high operating power which is considered energy-intensive and it also has risks of organic solvent explosion. Furthermore, exposure to microwave can cause considerable phenolic compounds degradation due to extreme high temperature (> 100 ºC) applied on certain parts of treated sample. Only PC with smaller size, such as gallic acid, quercetin and isoflavin, are known to be heat-stable up to 100 ºC for 20 min (Nn, 2015). Therefore, excessive microwave treatment can dramatically decrease PC extraction yield. Pressurized liquid extraction needs to be operated at high temperatures (up to 200 ºC) and high pressure (up to 300 psi) which is thus energy-consuming and also has the potential to degrade certain PC (Garcia-Salas et al., 2010; Medina-Torres et al., 2017). Furthermore, possible degradation of PC due to high frequency is the main disadvantage of ultrasound-assisted extraction.

Table 1.3 Alternative methods for PC extraction. Adapted from Aires (2017).

Method	Sample	Key findings	Reference
Soxhlet	*Centella asiatica*	Lower solvent usage	Nn, 2015
MAE	Blueberries (*Vaccinium corymbosum* L.)	Increased yield of anthocyanins	Routray & Orsat, 2014
	Grape seeds (*Vitis vinifera*)	Maximized extraction of antioxidant phenolics Lower solvent usage Shorter extraction time	Krishnaswamy et al., 2013
UAE	Leaves *(Cassia auriculate)*	Enhanced phenolics yield Shorter extraction time	Sharmila et al., 2016
	Mulberry pulp (*Morus nigra*)	Extracts with higher anthocyanin and total phenolic contents	Espada-Bellido et al., 2017
PLE	Mango (*Mangifera indica* L.)	Higher yields of polyphenols	Fernandez-Ponce et al., 2016
	Asparagus (*Asparagus officinalis* L)	Increased extraction efficiency of phenolic compounds Shorter extraction time Lower extraction temperature	Solana et al., 2015

1.3 Cold plasma

Plasma is often called the fourth state of matter after solid, liquid and gas, despite the fact that it is typically a partially ionized gas containing free electrons, ions, radicals, excited and non-excited molecules and photons. There are two types of plasmas in general, high-temperature and low-temperature plasmas, depending on the temperature of electrons and heavy species (neutrals and ions). The high-temperature plasma (e.g., fusion plasma) contains thermal equilibrium electrons and ions at temperatures higher than 10^7 K (Rutscher, 2008). The low-temperature plasma can be further divided into two types, thermal and nonthermal plasmas. Thermal plasma (e.g., arc plasma) has electrons and heavy species in a thermodynamic equilibrium state, and the temperature of electrons is much higher than that of heavy species in nonthermal plasma (Pankaj & Keener, 2017), such as glow discharge.

Nonthermal plasma is also called cold plasma, which is usually operated at temperatures lower than 40 ºC (Hoffmann et al., 2013). Cold plasma can be operated at low pressure or atmospheric pressure depending on its application. The atmospheric cold plasma does not require extreme operating conditions and costly reaction chamber to regulate pressure, which enables its potential applications for biological materials (Segat et al., 2016). There are various types of atmospheric cold plasma generation systems, including dielectric barrier discharge (DBD), plasma jet, corona discharge and microwave plasma (Misra et al., 2016). However, DBD and plasma jet are mostly used for food-related applications since they are easy to build up and more commercially available. DBD plasma is generated by two metal electrodes with at least one electrode covered with dielectric material (Figure 1.5). One electrode is the high voltage electrode and the other acts as the ground electrode. A carrier gas, such as air, argon, helium and nitrogen, moves between the two electrodes and is ionized to create plasma. The plasma jet

device consists of two concentric electrodes, and a gas flow is generated between them. The inner electrode is connected to a radio frequency power at a very high frequency (typically 13.56 MHz) to ionize the working gas. The ionized gas exits the nozzle and creates glow having a jet-like appearance (Misra et al., 2016).

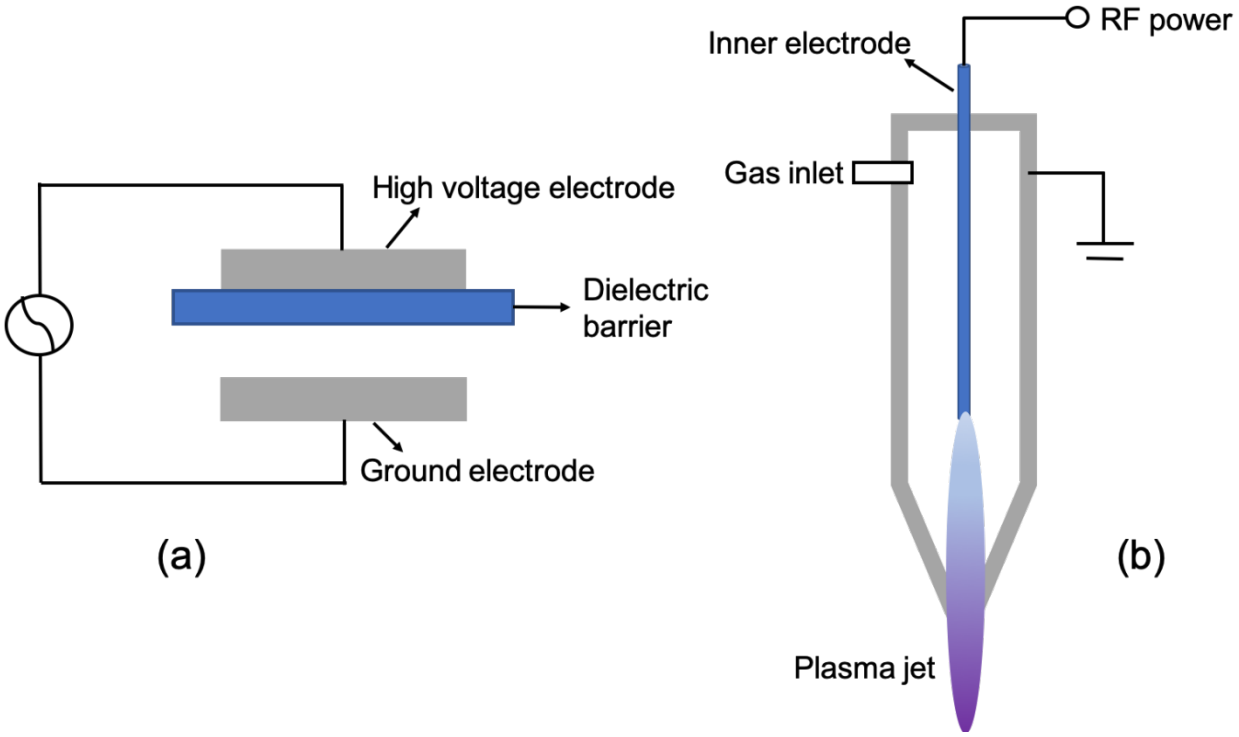

Figure 1.5 Schematic diagrams of (a) dielectric barrier discharge, and (b) plasms jet. Adapted from Misra et al. (2016).

Cold plasma can generate various types of reactive gas species, ultraviolet (UV) radiation, energetic ions, and charged particles that may induce physicochemical reactions to treated materials. These are influenced by the plasma type, applied voltage, working gas, treatment time, and relative humidity (Lotfy et al., 2020). For example, when discharging in air, mainly reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced, including ozone, hydrogen peroxide, singlet, atomic oxygen and NO_x species (Han et al., 2016). Different reactive species can be produced by using different working gases (Takamatsu et al., 2014). Argon,

helium and nirtogen plasmas can generate excited argon species, helium atoms and reactive nitrogen species, respectively. Moreover, processing time is another parameter affecting the reactive species generated. Gjika et al. (2018) found that the concentrations of H_2O_2 and NO_2^- continued increasing with extended plasma procesing time from 30 to 180 s.

Applications of cold plasma

Due to the unique characteristics described above, cold plasma has been applied in many areas, as shown in Table 1.4. Previously, cold plasma was mainly used in polymer industry as a method to modify and functionalize polymer surfaces. Cold plasma can increase the surface roughness and surface area of polymer fabrics through etching process (Poletti et al., 2003). Plasma etching is a process of material removal, in which the plasma-excited molecules collide with material surfaces at the atomic level (Nageswaran, Jothi & Jagannathan, 2019). These collisions can cause element removal and increase the roughness of treated surfaces. Changes in the surface morphology of materials can be analyzed with atomic force microscopy (AFM) and scanning electron microscopy (SEM), and quantified using the root-mean-square roughness value (R_{rms}) (Van Deynse et al., 2016). Cold plasma can also alter the surface wettability of polymers by changing both surface energy and surface O/C ratio and N/C ratio (Fatyeyeva et al., 2014). The C=O bond on the material surfaces can be converted to more hydrophilic C-O and C-OH bonds by plasma-generated reactive oxygen species, and the hydrophobic C-C and/or C-H bonds can be broken down by reactive nitrogen species. Moreover, plasma nitrogen species can also activate material surfaces, which can further react with excited nitrogen or oxygen to produce new hydrophilic functional groups, such as carboxyl, carbonate, amine and amide. The chemical compoistion and bonds of material surfaces can be characterized via spectroscopy, including X-ray photoelectron spectroscopy (XPS) and fourier transform infrared spectroscopy

(FTIR). XPS is usually used to analyze the top layer of materials (up to 10 nm), and FTIR is capable of penetrating into 600 nm below the material surfaces (Van Deynse et al., 2016). Those newly formed functional groups have marked effects on the surface free energy and surface wettability. When the surface energy continues to increase and exceeds the surface tension of the contacted liquid, the liquid droplet spreads out by the strong attractive force, indicating a more hydrophilic surface (Ruths, 2013). Surface wettability is determined by the contact angle of liquid droplet through the sessile drop method and surface energy can be calculated using the Owens-Wendt method (Owens & Wendt, 1969). Cold plasma-induced surface modifications are closely related to the properties of plasma (e.g., density, temperature, directed energies of charged particles), type of plasma attachment to surface (e.g., diffusive and filamentary) and type of discharge (e.g., corona, glow, arc) (Boxman, Sanders & Martin, 1995; Rossnagel, Westwood & Cuomo, 1990).

Table 1.4 Major applications of cold plasma. Adapated from Pankaj & Keener (2017).

Area	Application	Plasma source	Process parameter	Reference
Polymer	Sterilization	DBD	Frequency: 20-50 kHz; Power density: 7 W/cm^2; Gas: air, N_2 and Ar; Treatment time: 0−25 min	Heise et al., 2004
	Surface modification	RF plasma reactor	Frequency: 13.56 MHz; Power: 150 W; Gas: air, He, Ar, SF_6 and CF_4; Treatment time: 0−10 min	Poletti et al., 2003
	Surface functionalization	DBD	Frequency: 1-40 kHz; Voltage: 0−20 kV; Gas: H_2 and N_2; Treatment time: 0−200s	Sarra-Bournet et al., 2006
Medicine	Sterilization	Cold atmospheric surface micro-discharge plasma	Frequency: 1 kHz; Voltage: 10 kV; Power density: 35 mW/cm2; Gas: air; Treatment time: 1−5 min	Klämpfl et al., 2012
	Wound healing	KINPen plasma jet	Frequency: 1.0-1.1 MHz; Gas: argon	Bekeschus et al., 2016
	Disease treatment	Plasma jet	Frequency: 30 kHz; Voltage: 3-5 kV; Gas: He; Flow rate: 10−20 L/min; Treatment time: 0−120 s	Keidar et al., 2011
Water	Pesticide degradation	RF power plasma	Frequency: 13.56 MHz; Power: 30−120 W; Gas: oxygen; Treatment time: 30−120 s	Bai et al., 2010
	Dyes degradation	DBD	Frequency: 8000 Hz; Voltage: 30-50 kV; Gas: air; Flow rate: 14.5 L/min; Treatment time: 0−30 min	Tichonovas et al., 2013
	Decontamination	Dense medium plasma	Voltage: 100-300 V; Gas: oxygen; Treatment time: 0−115 s	Manolache, Shamamian & Denes, 2004
Food	Decontamination	HVACP	Voltage: 80 kV; Gas: air; Treatment time: 1−5 min	Han et al., 2016
	Enzyme inactivation	Cold atmospheric pressure plasma jet	Frequency: 1.1 MHz; Gas: argon + oxygen (0.01-0.1%); Flow rate: 5 L/min; Operation time: 0-360 s	Surowsky et al., 2013
	Toxin degradation	HVACP	Voltage: 90 kV; Gas: air and MA65 (65% O_2, 20% CO_2, 5% N_2); Treatment time: 1−30 min	Shi et al., 2017

With the ongoing development of plasma technology, its application has rapidly expanded to biomedical areas as a method to sterilize medical device, and treat certain diseases including wound healing, skin dentistry, cancer treatment, drug delivery, etc. (Fridman et al., 2008; Keidar et al., 2011; Shashurin et al., 2008).

Thermal processing, such as pasteurization and sterilization, is the most common practice to inactivate microorganisms in food products. However, associated processing techniques are usually energy-intensive and may also generate negative impacts on food products, such as losses of nutritional and sensory values (Misra et al., 2016). With operating temperatures (lower than 40 ºC) under the threshold of thermal damage to most biomaterials, cold plasma can better prevent the loss of heat-sensitive components in the treated food to preserve its quality and sensorial while being effective in microorganisms inactivation. The mechanisms of cold plasma-induced microbial inactivation are that plasma reactive species generate strong oxidative stress in microorganisms and cause cell damage and low-level DNA damage (Han et al., 2016). Cold plasma is also an effective method for inactivating unwanted enzymes in food by changing the secondary structure of enzyme (Surowsky et al., 2013). Other cold plasma applications in the field of food include toxin degradation (Shi et al., 2017), modification of food packaging materials (Pankaj et al., 2014), and nutritional value enhancement (Sainz-García et al., 2019).

1.4 Hypothesis and objectives of the study

Recovering natural bioactive compounds from fruit processing wastes, which are plentiful in the U.S. every year, is an economically viable strategy for their management. Considering the low extraction yield and high chemical usage of conventional extraction methods, a green and efficient alternative is needed. Due to the proven efficacy in surface modifications, cold plasma can be applied as a pretreatment on plant materials containing bioactive compounds, aiming to

facilitate the movement of hydrophilic compounds from intracellular matrix to surface during subsequent extraction, and thus increase the efficiency of conventional extraction.

This study is based on the hypothesis that cold plasma treatment can modify fruit pomace through: i) creating raptures on its cell wall structure, ii) further breaking the covalent bonds binding bioactive compounds, and iii) increasing its surface hydrophilicity. These modifications can help release bioactive compounds from plant cells and lower the hindrance to their migration, in order to increase their extractability for solvent extraction.

The main goal of this study is to develop an innovative cold plasma-assisted extraction process to valorize fruit pomace, as the source of bioactive compounds, into functional food ingredients and nutraceuticals with high nutritional and economic values, while helping reduce the waste generated by fruit processing. To test the hypothesis and thereby attain the main goal, this study includes three objectives: (i) examining the effect of DBD plasma, in terms of working gas and treatment time, on the surface microstructure and properties of fruit pomace, (ii) correlating surface modifications of fruit pomace with the extraction efficiency of their bioactive compounds, and (iii) analyzing the effect of cold plasma treatment on the bioactives composition and nutritional value of fruit pomace extracts.

References

Aires, A. (2017). Phenolics in Foods: Extraction, Analysis and Measurements. *Phenolic Compounds - Natural Sources, Importance and Applications*. https://doi.org/10.5772/66889

Arvanitoyannis, I. S., & Varzakas, T. (2008). *9 – Fruit/Fruit Juice Waste Management: Treatment Methods and Potential Uses of Treated Waste*. https://doi.org/10.1016/B978-012373654-3.50012-2

Babbar, N., Oberoi, H. S., Sandhu, S. K., & Bhargav, V. K. (2014). Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. *Journal of Food Science and Technology*, *51*(10), 2568–2575. https://doi.org/10.1007/s13197-012-0754-4

Bai, Y., Chen, J., Yang, Y., Guo, L., & Zhang, C. (2010). Degradation of organophosphorus pesticide induced by oxygen plasma: effects of operating parameters and reaction mechanisms. *Chemosphere*, *81*(3), 408-414.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food chemistry*, *99*(1), 191-203.

Barros, L., Dueñas, M., Pinela, J., Carvalho, A. M., Buelga, C. S., & Ferreira, I. C. (2012). Characterization and quantification of phenolic compounds in four tomato (Lycopersicon esculentum L.) farmers' varieties in northeastern Portugal homegardens. *Plant foods for human nutrition*, *67*(3), 229-234.

Bellik, Y., Boukraâ, L., Alzahrani, H. A., Bakhotmah, B. A., Abdellah, F., Hammoudi, S. M., & Iguer-Ouada, M. (2013). Molecular Mechanism Underlying Anti-Inflammatory and Anti-Allergic Activities of Phytochemicals: An Update. *Molecules*, *18*(1), 322–353. https://doi.org/10.3390/molecules18010322

Bekeschus, S., Schmidt, A., Weltmann, K. D., & von Woedtke, T. (2016). The plasma jet kINPen–A powerful tool for wound healing. *Clinical Plasma Medicine*, *4*(1), 19-28.

Boxman, R. L., Sanders, D. M., & Martin, P. J. (Ed.). (1995). *Handbook of Vacuum Arc Science and Technology*. Noyes Publications.

Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., & Bren, U. (2016). Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. *Molecules (Basel, Switzerland)*, *21*(7). https://doi.org/10.3390/molecules21070901

Cheng, A., Yan, H., Han, C., Chen, X., Wang, W., Xie, C., Qu, J., Gong, Z., & Shi, X. (2014). Acid and Alkaline Hydrolysis Extraction of Non-Extractable Polyphenols in Blueberries: Optimisation by Response Surface Methodology. *Czech J. Food Sci.*, *32*(3), 8.

Espada-Bellido, E., Ferreiro-González, M., Carrera, C., Palma, M., Barroso, C. G., & Barbero, G. F. (2017). Optimization of the ultrasound-assisted extraction of anthocyanins and total phenolic compounds in mulberry (Morus nigra) pulp. *Food chemistry*, *219*, 23-32.

Fatyeyeva, K., Dahi, A., Chappey, C., Langevin, D., Valleton, J. M., Poncin-Epaillard, F., & Marais, S. (2014). Effect of cold plasma treatment on surface properties and gas permeability of polyimide films. *Rsc Advances*, *4*(59), 31036-31046.

Fernandez-Ponce, M. T., Parjikolaei, B. R., Lari, H. N., Casas, L., Mantell, C., & de la Ossa, E. J. M. (2016). Pilot-plant scale extraction of phenolic compounds from mango leaves using different green techniques: Kinetic and scale up study. *Chemical Engineering Journal*, *299*, 420-430.

Fridman, G., Friedman, G., Gutsol, A., Shekhter, A. B., Vasilets, V. N., & Fridman, A. (2008). Applied Plasma Medicine. *Plasma Processes and Polymers*, *5*(6), 503–533. https://doi.org/10.1002/ppap.200700154

Garcia, E., & Barrett, D. M. (2006). Peelability and yield of processing tomatoes by steam or lye. *Journal of food processing and preservation*, *30*(1), 3-14.

Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Phenolic-compound-extraction systems for fruit and vegetable samples. *Molecules (Basel, Switzerland)*, *15*(12), 8813–8826. https://doi.org/10.3390/molecules15128813

Gerszberg, A., Hnatuszko-Konka, K., Kowalczyk, T., & Kononowicz, A. K. (2015). Tomato (Solanum lycopersicum L.) in the service of biotechnology. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *120*(3), 881-902.

Giada, M. D. L. R. (2013). Food phenolic compounds: main classes, sources and their antioxidant power. *Oxidative stress and chronic degenerative diseases—A role for antioxidants. InTech*, 87-112.

Gjika, E., Pal-Ghosh, S., Tang, A., Kirschner, M., Tadvalkar, G., Canady, J., ... & Keidar, M. (2018). Adaptation of operational parameters of cold atmospheric plasma for in vitro treatment of cancer cells. *ACS applied materials & interfaces*, *10*(11), 9269-9279.

Gould, W. A. (1992). CHAPTER 10—Tomato Pulp and Paste Manufacture. In W. A. Gould (Ed.), *Tomato Production, Processing and Technology* (pp. 219–231). Woodhead Publishing. https://doi.org/10.1533/9781845696146.2.219

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P. J., & Bourke, P. (2016). Mechanisms of Inactivation by High-Voltage Atmospheric Cold Plasma Differ for Escherichia coli and Staphylococcus aureus. *Applied and Environmental Microbiology*, *82*(2), 450–458. https://doi.org/10.1128/AEM.02660-15

Heise, M., Neff, W., Franken, O., Muranyi, P., & Wunderlich, J. (2004). Sterilization of polymer foils with dielectric barrier discharges at atmospheric pressure. *Plasmas and polymers*, *9*(1), 23-33.

Hoffmann, C., Berganza, C., & Zhang, J. (2013). Cold Atmospheric Plasma: Methods of production and application in dentistry and oncology. *Medical Gas Research*, *3*, 21. https://doi.org/10.1186/2045-9912-3-21

Iannone, R., Miranda, S., Riemma, S., & De Marco, I. (2016). Improving environmental performances in wine production by a life cycle assessment analysis. *Journal of Cleaner Production*, *111*, 172-180.

Jideani, A. I. O., Anyasi, T. A., Mchau, G. R. A., O.Udoro, E., & Onipe, O. O. (2017). Processing and Preservation of Fresh-Cut Fruit and Vegetable Products. *Postharvest Handling*. https://doi.org/10.5772/intechopen.69763

Keidar, M., Walk, R., Shashurin, A., Srinivasan, P., Sandler, A., Dasgupta, S., Ravi, R., Guerrero-Preston, R., & Trink, B. (2011). Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. *British Journal of Cancer*, *105*(9), 1295–1301. https://doi.org/10.1038/bjc.2011.386

Kelley, W. T., Boyhan, G. E., Harrison, K. A., Sumner, P. E., Langston, D. B., Sparks, A. N., Culpepper, S., Hurst, W. C., & Fonsah, E. G. (2010). *Commercial tomato production handbook*.

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328-2375.

Klämpfl, T. G., Isbary, G., Shimizu, T., Li, Y. F., Zimmermann, J. L., Stolz, W., ... & Schmidt, H. U. (2012). Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. *Appl. Environ. Microbiol.*, *78*(15), 5077-5082.

Krishnaswamy, K., Orsat, V., Gariépy, Y., & Thangavel, K. (2013). Optimization of microwave-assisted extraction of phenolic antioxidants from grape seeds (Vitis vinifera). *Food and Bioprocess Technology*, *6*(2), 441-455.

Lasik, M., Majcher, M., & Nowak, J. (2018). Effects of Different Techniques of Malolactic Fermentation Induction on Diacetyl Metabolism and Biosynthesis of Selected Aromatic Esters in Cool-Climate Grape Wines. *Molecules*, *23*, 2549. https://doi.org/10.3390/molecules23102549

Lieu, L. N., & Le, V. V. M. (2010). Application of ultrasound in grape mash treatment in juice processing. *Ultrasonics-Sonochemistry*, *17*(1), 273-279.

Lotfy, K., Khalil, S. M., & El-Raheem, H. A. (2020). Inactivation by helium cold atmospheric pressure plasma for Escherichia coli and Staphylococcus aureus. *Journal of Theoretical and Applied Physics*, *14*(1), 37–45. https://doi.org/10.1007/s40094-019-00362-4

Markets and Markets. (2015). Phytonutrients Market by Type (Carotenoids, Phytosterols, Flavonoids, Phenolic Compounds, and Vitamin E), Application (Food & Beverage, Feed, Pharmaceutical, and Cosmetic), Source, & by Region - Global Trends and Forecast to 2020. Retrieved from https://www.marketsandmarkets.com/Market-Reports/phytonutrients-market-1101.html

Market Research Report. (2019). Anthocyanin Market Analysis, Market Size, Application Analysis, Regional Outlook, Competitive Strategies, And Segment Forecasts, 2015 to 2022. Retrieved from https://www.grandviewresearch.com/industry-analysis/anthocyanin-market

Madhujith, T., & Shahidi, F. (2009). Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chemistry*, *117*(4), 615–620. https://doi.org/10.1016/j.foodchem.2009.04.055

Manolache, S., Shamamian, V., & Denes, F. (2004). Dense medium plasma-plasma-enhanced decontamination of water of aromatic compounds. *Journal of environmental engineering*, *130*(1), 17-25.

Martínez-Valverde, I., Periago, M. J., Provan, G., & Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture*, *82*(3), 323-330.

Medina-Torres, N., Ayora-Talavera, T., Espinosa-Andrews, H., Sánchez-Contreras, A., & Pacheco, N. (2017). Ultrasound Assisted Extraction for the Recovery of Phenolic Compounds from Vegetable Sources. *Agronomy*, *7*(3), 47. https://doi.org/10.3390/agronomy7030047

Misra, N. N., Pankaj, S. K., Segat, A., & Ishikawa, K. (2016). Cold plasma interactions with enzymes in foods and model systems. *Trends in Food Science & Technology*, *55*, 39–47. https://doi.org/10.1016/j.tifs.2016.07.001

Mokhtarpour, A., Naserian, A. A., Valizadeh, R., Mesgaran, M. D., & Pourmollae, F. (2014). Extraction of Phenolic Compounds and Tannins from Pistachio By-products. *Annual Research & Review in Biology*, 1330–1338. https://doi.org/10.9734/ARRB/2014/7793

Mosca, F., Hidalgo, G. I., Villasante, J., & Almajano, M. P. (2018). Continuous or batch solid-liquid extraction of antioxidant compounds from seeds of Sterculia apetala plant and kinetic release study. *Molecules*, *23*(7), 1759.

Nageswaran, G., Jothi, L., & Jagannathan, S. (2019). Plasma Assisted Polymer Modifications. In *Non-Thermal Plasma Technology for Polymeric Materials* (pp. 95-127). Elsevier.

Nn, A. (2015). *A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation*. https://doi.org/10.4172/2167-0412.1000196

OECD. (2016). Chapter 2: Tomato (*Solanum lycopersicum*). *Harmonisation of Regulatory Oversight in Biotechnology Safety Assessment of Transgenic Organisms in the Environment, Volume 6: OECD Consensus Documents*. OECD.

Owens, D. K., & Wendt, R. C. (1969). Estimation of the surface free energy of polymers. *Journal of applied polymer science*, *13*(8), 1741-1747.

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., Milosavljević, V., O'donnell, C. P., Bourke, P., ... & Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, *35*(1), 5-17.

Pankaj, S. K., & Keener, K. M. (2017). Cold plasma: Background, applications and current trends. *Current Opinion in Food Science*, *16*, 49–52. https://doi.org/10.1016/j.cofs.2017.07.008

Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of Cold Plasma on Food Quality: A Review. *Foods*, *7*(1), 4. https://doi.org/10.3390/foods7010004

Poletti, G., Orsini, F., Raffaele-Addamo, A., Riccardi, C., & Selli, E. (2003). Cold plasma treatment of PET fabrics: AFM surface morphology characterisation. *Applied Surface Science*, *219*(3), 311–316. https://doi.org/10.1016/S0169-4332(03)00703-7

Reisch, B. I., Owens, C. L., & Cousins, P. S. (2012). Grape. In *Fruit breeding* (pp. 225-262). Springer, Boston, MA.

Rossnagel, S. M., Westwood, W. D., & Cuomo, J. J. (Ed.). (1990). *Handbook of Plasma Processing Technology*. Noyes Publications.

Routray, W., & Orsat, V. (2014). MAE of phenolic compounds from blueberry leaves and comparison with other extraction methods. *Industrial Crops and Products*, *58*, 36-45.

Ruths M. (2013) Surface Forces, Surface Tension, and Adhesion. In: Wang Q.J., Chung YW. (eds) Encyclopedia of Tribology. Springer, Boston, MA.

Rutscher, A. (2008). Characteristics of low-temperature plasmas under nonthermal conditions–a short summary. *Low Temperature Plasmas: Fundamentals, Technologies and Techniques, 1,* 1-14.

Sainz-García, E., López-Alfaro, I., Múgica-Vidal, R., López, R., Escribano-Viana, R., Portu, J., ... & González-Arenzana, L. (2019). Effect of the Atmospheric Pressure Cold Plasma Treatment on Tempranillo Red Wine Quality in Batch and Flow Systems. *Beverages*, *5*(3), 50.

Sarra-Bournet, C., Turgeon, S., Mantovani, D., & Laroche, G. (2006). A study of atmospheric pressure plasma discharges for surface functionalization of PTFE used in biomedical applications. *Journal of Physics D: Applied Physics*, *39*(16), 3461.

Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2016). Effect of atmospheric pressure cold plasma (ACP) on activity and structure of alkaline phosphatase. *Food and Bioproducts Processing*, *98*, 181–188. https://doi.org/10.1016/j.fbp.2016.01.010

Sellappan, S., Akoh, C. C., & Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of agricultural and food chemistry*, *50*(8), 2432-2438.

Sharmila, G., Nikitha, V. S., Ilaiyarasi, S., Dhivya, K., Rajasekar, V., Kumar, N. M., ... & Muthukumaran, C. (2016). Ultrasound assisted extraction of total phenolics from Cassia auriculata leaves and evaluation of its antioxidant activities. *Industrial crops and Products*, *84*, 13-21.

Shashurin, A., Keidar, M., Bronnikov, S., Jurjus, R. A., & Stepp, M. A. (2008). Living tissue under treatment of cold plasma atmospheric jet. *Applied Physics Letters*, *93*(18), 181501. https://doi.org/10.1063/1.3020223

Shi, H., Ileleji, K., Stroshine, R. L., Keener, K., & Jensen, J. L. (2017). Reduction of Aflatoxin in Corn by High Voltage Atmospheric Cold Plasma. *Food and Bioprocess Technology*, *10*(6), 1042–1052. https://doi.org/10.1007/s11947-017-1873-8

Solana, M., Boschiero, I., Dall'Acqua, S., & Bertucco, A. (2015). A comparison between supercritical fluid and pressurized liquid extraction methods for obtaining phenolic compounds from Asparagus officinalis L. *The Journal of Supercritical Fluids*, *100*, 201-208.

Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering*.

Su, D., Zhang, R., Hou, F., Zhang, M., Guo, J., Huang, F., Deng, Y., & Wei, Z. (2014). Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. *BMC Complementary and Alternative Medicine*, *14*, 9. https://doi.org/10.1186/1472-6882-14-9

Surowsky, B., Fischer, A., Schlueter, O., & Knorr, D. (2013). Cold plasma effects on enzyme activity in a model food system. *Innovative Food Science & Emerging Technologies*, *19*, 146–152. https://doi.org/10.1016/j.ifset.2013.04.002

Takamatsu, T., Uehara, K., Sasaki, Y., Miyahara, H., Matsumura, Y., Iwasawa, A., ... & Okino, A. (2014). Investigation of reactive species using various gas plasmas. *RSC Advances*, *4*(75), 39901-39905.

Tichonovas, M., Krugly, E., Racys, V., Hippler, R., Kauneliene, V., Stasiulaitiene, I., & Martuzevicius, D. (2013). Degradation of various textile dyes as wastewater pollutants under dielectric barrier discharge plasma treatment. *Chemical Engineering Journal*, *229*, 9-19.

Tsao, R. (2010). Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*, *2*(12), 1231–1246. https://doi.org/10.3390/nu2121231

United States Department of Agriculture - National Agricultural Statistics Service. (2019). Noncitrus Fruits and Nuts 2018 Summary.

USDA ERS - Fruit & Tree Nuts. (n.d.). Retrieved from https://www.ers.usda.gov/topics/crops/fruit-tree-nuts/

Van Deynse, A., Morent, R., & De Geyter, N. (2016). Surface modification of polymers using atmospheric pressure cold plasma technology. In *Polymer science: Research advances, pratical applications and educational aspects* (pp. 506–516). Formatex Research Center.

Ventura, M. R., Pieltain, M. C., & Castanon, J. I. R. (2009). *Evaluation of tomato crop by-products as feed for goats*.

Yahia, E. M. (Ed.). (2017). *Fruit and Vegetable Phytochemicals: Chemistry and Human Health, 2 Volumes*, Chapter 6. John Wiley & Sons.

CHAPTER 2. NOVEL COLD PLASMA-ASSISTED EXTRACTION FOR PHENOLIC COMPOUNDS FROM TOMATO POMACE

Yiwen Bao[a], Lavanya Reddivari[a], Jen-Yi Huang[a,b,*]

[a]Department of Food Science, Purdue University, West Lafayette, IN, USA

[b]Environmental and Ecological Engineering, Purdue University, West Lafayette, IN, USA

*Corresponding author. Tel.: +1-765-496-6034, Fax: +1-765-494-7953

Address: 745 Agriculture Mall Drive, West Lafayette, IN 47907, USA

E-mail: huang874@purdue.edu

Submitted to

Innovative Food Science and Emerging Technologies

April 2020

Abstract

Tomato pomace (TP) is a good source of phenolic compounds (PC), however, the huge amount generated from commercial tomato processing has caused a severe waste problem. Conventional extraction methods for PC are generally time-consuming with intensive uses of organic solvents that are thus considered not environment-friendly. With the proven efficacy in material modification, high voltage atmospheric cold plasma (HVACP) was used in this study to modify the TP surface, aiming to facilitate the PC migration during extraction hence increase their extraction yields. Here, the effects of HVACP treatments (air, Ar, He and N_2) on the TP surface properties, and PC extraction efficiency, compositions, and antioxidant activity were studied. In addition to rupturing TP epidermal cells, HVACP treatments were found to decrease the water contact angles of tomato peels and accelerate the drying of tomato fruit, indicating that the treated surfaces were more hydrophilic. TP treated by He and N_2 plasmas showed increased PC extraction yields by nearly 10%, and all HVACP treatments increased the antioxidant activity of PC extracts and slightly changed their compositions. This novel HVACP-assisted extraction technology successfully improved the extraction efficiency and antioxidant activity of PC from TP, which is a promising method for utilizing tomato processing byproducts with high nutritional and economic values.

Keywords: *High voltage atmospheric cold plasma; Extraction enhancement; Surface modification; Bioactive compounds; Tomato byproducts; Antioxidant activity*

Highlights:

- Cold plasma treatments enhance PC extraction from TP through surface modifications
- HVACP ruptures TP cell wall structures
- HVACP makes tomato peel more hydrophilic and accelerates tomato drying
- Extracts from HVACP-treated TP have higher TPC and AA, and different PC profiles

2.1 Introduction

Tomato is one of the most popular crops in the U.S., with annual production of over 14 million tons, in which almost 86% are processed to different products (Kelley et al., 2010). Commercial tomato processing can generate 5−10% tomato pomace (TP; mainly peels and seeds), which has caused a severe waste problem (Ventura et al., 2009). This huge amount of byproduct is normally used to feed animals or distributed to landfill directly, ending up with low values. Therefore, the management of TP has become an important problem for the tomato processing industry (Bennamoun et al., 2016).

As the secondary metabolites, phenolic compounds (PC) are widely present in plants, which have great nutritional benefits to human health, such as decreasing the risk of degenerative diseases by reducing oxidative stress and inhibiting macromolecular oxidation (Lin et al., 2016). TP is a good source of PC with a relative high content of 73 mg/100 g (Knoblich et al., 2005). Hence, TP can be valorized through recovery of PC. In TP, PC can be in either free form or bound form. The extraction of bound PC is difficult since it is hindered by the cell wall structure which may even entrap the free PC. Therefore, the choice of extraction method can greatly influence the PC extraction yield (Khoddami et al., 2013).

The conventional methods for PC extraction are liquid-liquid extraction or solid-liquid extraction. However, there are two main concerns with these processes: intensive solvent use (e.g., methanol, ethanol, acetone, etc.) and relatively low extraction efficiency (Aspé & Fernández, 2011). Besides, conventional extraction methods are not effective in extracting bound PC from plant matrix (Su et al., 2014). In order to improve the extractability of bound PC, alkaline or acid hydrolysis is normally used (Cheng et al., 2014; Madhujith & Shahidi, 2009), which is not an environment-friendly approach either because of large demand for strong acid/alkaline, as well as operation at extremely high temperatures (up to 80–95 ºC for acid hydrolysis) (Garcia-Salas et al., 2010). Therefore, a green and effective alternative extraction method is an emerging need.

Cold plasma is a type of plasma typically generated at an atmospheric pressure with low energy cost while converting gaseous medium to a highly reacting state. It can generate various types of reactive gas species, UV radiation, energetic ions, and charged particles that may induce physicochemical reactions with treated materials (Hoffmann et al., 2013). These reactions are influenced by the plasma type, applied voltage, working gas, treatment time, and relative humidity (Lotfy et al., 2020). The exposure to plasma reactive species has been widely used for modification of various material surfaces, including increasing surface roughness and surface area through etching process (Poletti et al., 2003), modulating wettability of polymer surfaces (Van Deynse et al., 2016), etc. For food applications, cold plasma has been used for commercial sterilization, in which plasma reactive species attack the cell envelope of bacteria, inducing its disruption and resulting in cell leakage (Han et al., 2016). Cold plasma also has the capability of manipulating surface characteristics of food products. The surfaces of fresh lettuce and white grape became more hydrophilic after plasma treatments due to degradation of the cuticle layer,

which consists of hydrophobic cuticular and epicuticular waxes (Grzegorzewski et al., 2010; Huang et al., 2019). The cellular damages and surface modifications caused by plasma indicate its potential for decreasing the resistance to diffusion of internal molecules and increasing the extractability of hydrophilic compounds, which can facilitate PC recovery from biomass. However, to the best of our knowledge, current application of cold plasma towards nutrients extraction has been only explored by one study on essential oil (Kodama et al., 2014), and it has not been applied for extraction of PC so far. Moreover, cold plasma treatment has shown inconsistent effects on the total phenolic content (TPC) of food products. Cold plasma was found to decrease the TPC in orange juice (Almeida et al., 2015) and white grape juice (Pankaj et al., 2017), but enhance the TPC in cashew apple juice (Rodríguez et al., 2017). This highlights the research gap that the interaction between plasma reactive species and PC at molecular level is unknown.

Based on the proven capabilities of cold plasma for modifying biomass through creating raptures on its cell wall structure and increasing its surface hydrophilicity, in this study, we developed a novel cold plasma-assisted extraction method for PC from TP. The objectives of this study were to examine the effect of cold plasma treatment on the microstructure of TP and correlate its surface modification with PC extraction efficiency.

2.2 Materials and methods

2.2.1 Materials

TP was collected from the tomato processing facility of Red Gold (Orestes, IN, USA). To better characterize the effect of cold plasma on the sample hydrophilicity, tomato samples were treated to analyze the surface wettability of tomato peel and the drying kinetics of tomato fruit.

Greenhouse tomatoes and cherry tomatoes were purchased from the Walmart Supercenter (West Lafayette, IN, USA).

Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagents were purchased from Fisher Scientific (Pittsburgh, PA, USA). PC standards including chlorogenic acid, caffeic acid, trans-ferulic acid, rutin, isoquercetin, quercetin and naringenin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents for ultra-performance liquid chromatography (UPLC) analysis were purchased from VWR International (Bristol, CT, USA).

2.2.2 Sample preparation

TP was freeze-dried for 48 h after collection. Dried TP was blended then filtered with a 20-mesh sieve to prepare the powder sample that was stored at −20 ºC. For the plasma treatment, TP powder (0.5 g) was homogeneously distributed at the bottom of a glass petri dish (8 cm diameter) covered with parafilm. The petri dish was then flushed by the working gas of plasma treatment before sealed by another layer of parafilm. To measure the change in surface wettability, pieces of tomato peel (2 × 2 cm) were cut from greenhouse tomato for plasma treatment. The tomato peel or whole cherry tomatoes (for drying test) were placed in a polypropylene box (27.9 × 18.4 × 4.4 cm), and the box was sealed in a plastic bag of high barrier film (B2630T, Cryovac® Sealed Air Inc, NJ, USA) after flushing by the working gas of plasma treatment.

2.2.3 High voltage atmospheric cold plasma treatment

Figure 2.1 shows the schematic of the high voltage atmospheric cold plasma (HVACP) system used in this study, which included a dielectric barrier discharge (DBD) setup powered by a Phenix BK-130 transformer (Phenix Technologies, MD, USA) with an input voltage of 120 V

(AC) at 60 Hz (Suwal et al., 2019; Xu et al., 2017). The sample container/package was placed between two circular-shaped aluminum electrodes (15.24 cm diameter) with a gap of 5.2 cm. Polypropylene pads (355 × 272 × 2.20 mm, Cuisinart, NJ, USA) were placed above and below the container/package as the additional dielectric barriers to ensure more uniform plasma discharge. The sample was exposed to the plasma generated by different working gases (air, argon, helium and nitrogen) under 60 kV for 15 min then kept for further analyses.

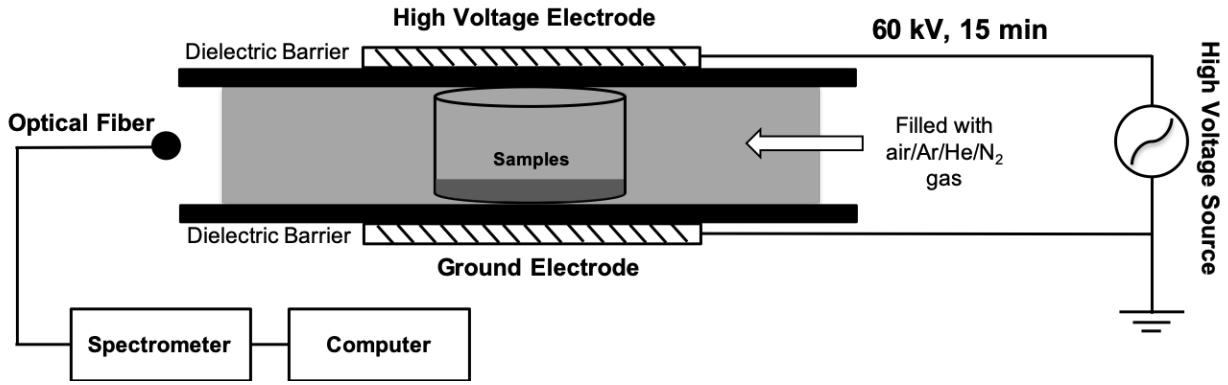

Figure 2.1 Schematic diagram of the experimental setup for high voltage atmospheric cold plasma (HVACP) treatment and optical emission spectroscopy (OES) analysis.

2.2.4 Optical emission spectroscopy

Optical emission spectroscopy (OES) was used to characterize the reactive gas species generated during HVACP treatment using a HR2000+ spectrometer connected with an optical fiber (Ocean Optics, Largo, FL, USA). A 200−2000 nm UV-VIS collimating lens was set on the fiber and placed 140 mm away from plasma discharge to capture the emission light. Data was recorded every 30 s using the OceanView software.

2.2.5 Surface microstructure characterization

The surface microstructure of the TP was identified using scanning electron microscopy (SEM). TP powder was mounted on carbon tape and sputter-coated with platinum for 60 s.

Images were taken by FEI Quanta 3D FEG (FEI Company, OR, USA) at an accelerating voltage of 5 kV.

2.2.6 Surface wettability determination

The surface wettability of tomato peel was determined using a droplet shape analyzer (KRÜSS DSA 30, Hamburg, Germany) by measuring its surface-water contact angle according to the sessile drop method (Gilbert et al., 2018). The contact angle of the tomato peel pieces was measured at room temperature within 10 mins after HVACP treatment. Specifically, a liquid drop of distilled water (approximately 8 μL) was dispersed on the waxy surface of the tomato peel piece. The average contact angle was determined based on four measurements.

2.2.7 Phenolic compounds extraction

PC were extracted from TP following Han et al. (2018) with modifications. TP powder (0.3 g) was placed in a 50-mL centrifuge tube and well mixed with 12 mL of 50% (v/v) ethanol under vortex for 30 s. The mixture was stirred using an incubator shaker at 150 rpm at room temperature for 15 min before centrifugation at 2000 × g for 15 min. The supernatant was collected and concentrated under nitrogen flow. The concentrated extract was then freeze-dried and reconstituted with 3 mL of 50% ethanol.

2.2.8 Total phenolic content determination

The TPC of the TP extract was determined by the Folin-Ciocalteu micro-method (Slinkard & Singleton, 1977). Thirty-five microliters of phenolic extract were mixed with 150 μL diluted Folin-Ciocalteu's reagent (10-fold) under continuous shaking at 400 rpm for 30 s. After incubation at room temperature for 5 min, 115 μL of 7.5% Na2CO3 (w/v) solution was added

and shaken (400 rpm) for 30 s. The mixture was then incubated at 45 ºC for 30 min, followed by additional 1 h incubation at room temperature in dark. The absorbance of the mixture was then measured by a Cytation 1 multi-mode reader (BioTek Instruments Inc., Winooski, VT, USA) at 765 nm wavelength. The TPC was expressed as gallic acid equivalents (GAE). A standard curve of gallic acid was established with a concentration gradient from 40 to 200 μg/mL.

2.2.9 Antioxidant activity determination

The DPPH radical-scavenging activity was measured to determine the antioxidant capacity of the TP extracts (Brand-Williams et al., 1995). TP extract (15 μL) was added into 285 μL of DPPH solution, then the mixture was shaken at 400 rpm for 30 s. Decolorization was determined after 2 h of incubation in dark by measuring the absorbance at 515 nm. A standard curve of trolox was established with a concentration gradient from 40 to 200 μg/mL.

2.2.10 Phenolic composition identification

The phenolic composition of the TP extract was identified and quantified using a Waters Acquity H-Class UPLC system (Milford, MA, USA) equipped with a photodiode array detector (PAD). Following Li et al. (2016), the Waters BEH C18 column (2.1 × 100 mm; 1.7 μm) was used to separate individual phenolic compounds at 40 ºC with a flow rate of 0.5 mL/min. The sample injection volume was 10 μL. Mobile phase A contained 0.2% formic acid in acetonitrile (ACN) and mobile phase B was 0.2% formic acid in water/ACN (95/5). The column was eluted following a gradient system: 0 min, 100% B; 3 min, 91% B; 3−7 min, 91−68% B; 8 min, 100% B; 8−10 min, 100% B. Chromatographs were extracted at 290 nm for phenolic acids and 350 nm for flavonoids. PC were identified by spiking and comparing retention times and UV spectra

with the standards. The amount of each PC was quantified according to the standard curves for gallic acid, chlorogenic acid, caffeic acid, trans-ferulic acid, rutin, isoquercetin, quercetin and naringenin.

All the PC were also confirmed using a triple quadrupole mass spectrometer (Agilent 6460; Santa Clara, CA, USA) coupled with rapid resolution high performance liquid chromatography (Agilent 1200) by the targeted metabolomics approach. Five microliters of sample were injected and separated by an XBridge™ C18 column (2.1 × 100 mm, 3.5 μm) at the flow rate of 0.35 mL/min. The column was eluted with a gradient of 0.2% formic acid in water/ACN (95/5) (solvent A) and 0.2% formic acid in ACN (solvent B) following: 0−1 min, 100% A; 1−7 min, 100−5% A; 7−10 min, 5% A; 10.1 min, 100% A; 10.1−14 min, 100% A.

2.2.11 Statistical analysis

All the experiments were performed in triplicate. Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test ($p < 0.05$) to assess group differences using SPSS v23.0 (SPSS Inc., Chicago, IL, USA).

2.3 Results and discussion

2.3.1 OES analysis

Spectra emissions of different reactive gas species generated by HVACP were characterized continuously by OES, as shown in Figure 2.2. N_2 (315.9, 337.1, 357.7, 380.5, 405.9, 427.8 nm), OH (309 nm) and O (616 and 780 nm) are the major species present in air plasma (Xu et al., 2017), however, the only predominant peaks shown here (Figure 2.2a) were associated with N_2 species transition and there were no OH species and O atom observed. This was because OH and

O_2 species have weaker peaks and the former can easily overlap with N_2 peaks (Shi et al., 2017). Therefore, the nitrogen plasma showed a similar spectrum (Figure 2.2d) to the air plasma but with higher intensity of N_2 species due to higher nitrogen concentration. For the argon plasma (Figure 2.2b), the spectrum emitted was dominated by the Ar peaks, starting from the wavelength of 690 nm (Hoentsch et al., 2014). Figure 2.2c shows the spectrum of the helium plasma with several emission peaks (390.4, 587.0, 501.3, 667.3, 705.8 and 727.9 nm) representing He atom (Zhang et al., 2015). It is important to note that the spectra of helium and argon plasmas also showed N_2 and N_2^+ emission peaks, indicating the air residue remaining in the plasma chamber. This suggests that the helium, argon and nitrogen plasmas tested in this study included certain amounts of reactive oxygen and nitrogen species generated through ionization of air residue, which also participated in the treatments on the TP and tomato samples.

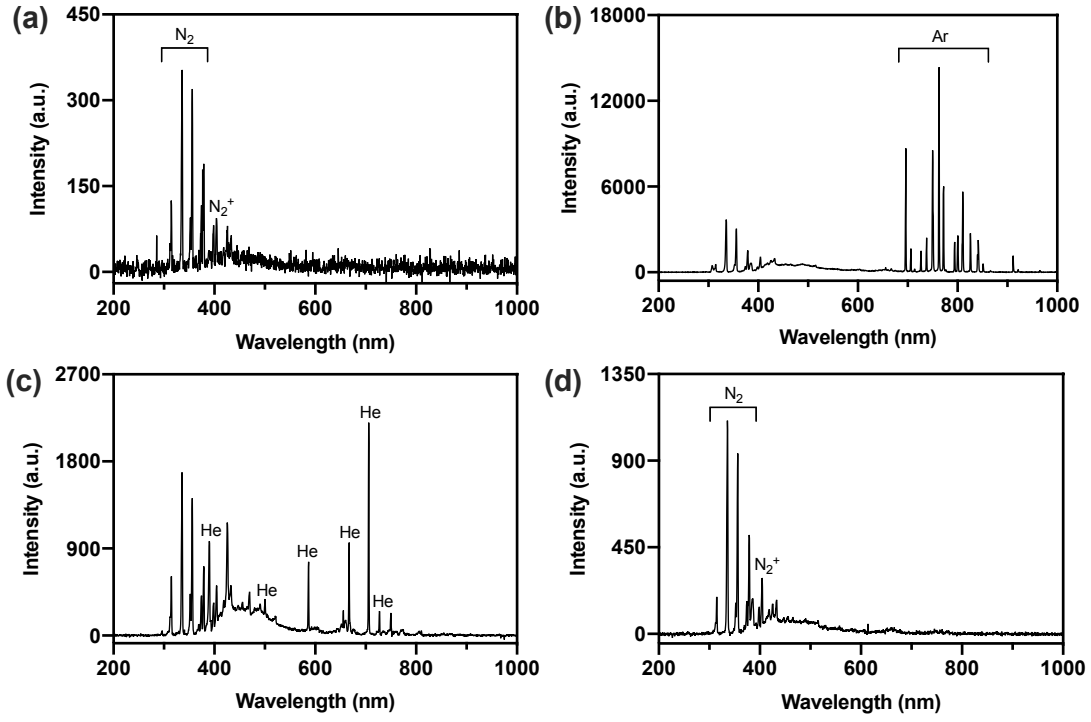

Figure 2.2 Optical emission spectra of HVACP at 60 kV for (a) air, (b) argon, (c) helium, (d) nitrogen.

2.3.2 Effect of HVACP on surface microstructure

Figure 2.3 shows the surface microstructure of both HVACP-treated and control TP under SEM. The control TP displayed the epidermal cells with clearly defined contours of cell wall structures (Figure 2.3a). After HVACP treatments, the samples showed less or no visible cell wall structures and lost their cell integrity. The argon plasma treatment caused the most intensive disruption in the TP cell structures through breaking down the cell wall into fragments (Figure 2.3c). This agreed with the emission peaks of higher intensity (i.e., larger amount of plasma reactive species) observed with the argon plasma (Figure 2.2b), which resulted in a greater level of modification on the TP surface microstructure.

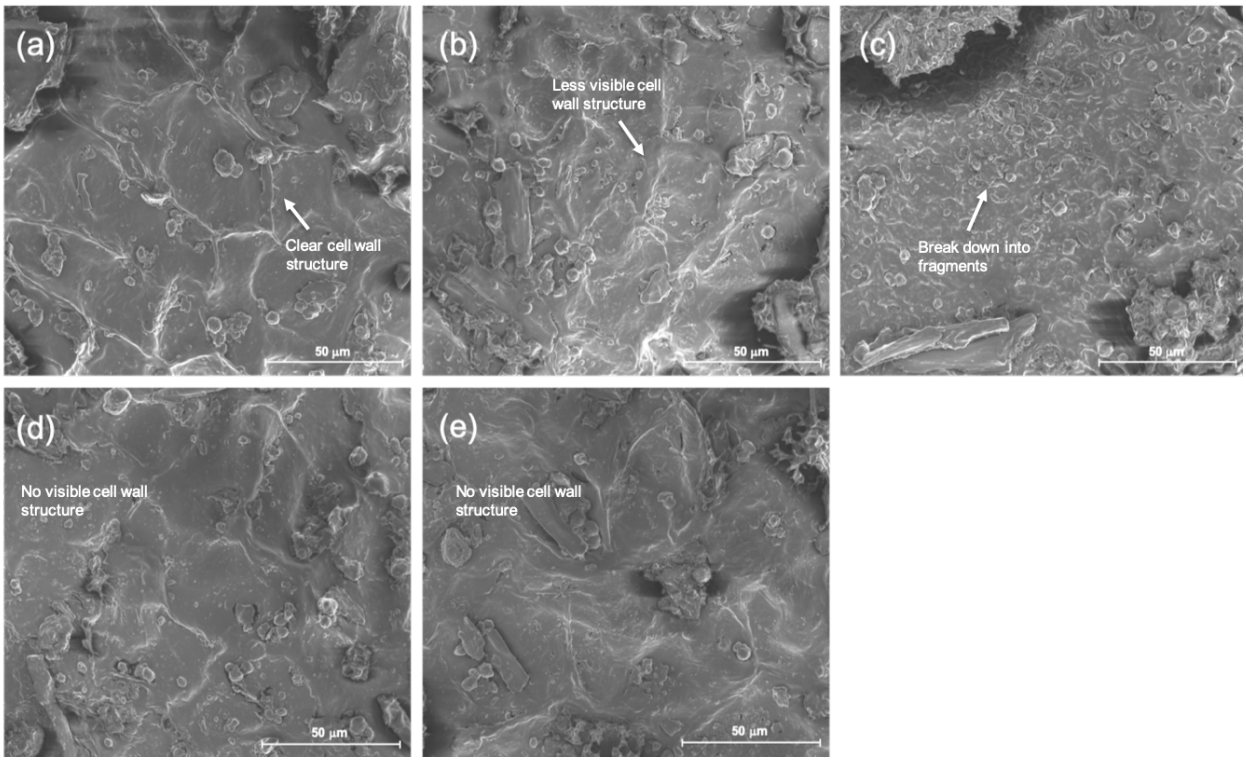

Figure 2.3 SEM images of tomato pomace treated by HVACP at 60 kV for 15 min: (a) control, (b) air, (c) argon, (d) helium, (e) nitrogen.

Similar findings were reported by Seol et al. (2017) that the treatment of 5-kV helium plasma jet for 6 s disrupted the epidermis structure of cotyledon which was indicated by weaker cell wall signals under confocal microscope. Other types of changes in the surfaces of plasma-treated fruit and vegetable were also reported. Kodama et al. (2014) found a damaged and scorched lemon peel surface after DBD plasma treatment at 30 kV. Huang et al. (2019) observed numerous surface cracks and waxy layer dissociation of white grape treated by atmospheric plasma jet at a power of 500 W. Grzegorzewski et al. (2010) noticed tough and bumpy surface in lettuce after exposure to low-pressure oxygen plasma at 150 W for 60 s. In addition to cell wall structure, plasma reactive species can disrupt cell membrane integrity and lead to cell leakage, which has been regarded as one of the mechanisms behind microbial inactivation (L. Han et al., 2016; Joshi et al., 2011).

2.3.3 Effect of HVACP treatment on surface wettability

Figure 2.4 displays the water contact angles of tomato peel samples. While the control had a more hydrophobic surface (88º) because of the waxy layer, significant decreases in the contact angle of the samples by 11.4−31.8% (i.e., more hydrophilic) can be observed with HVACP treatment, regardless of the working gas. Among the working gases tested, argon and helium showed the greatest enhancement in surface wettability with the values of 60º and 64º, respectively. Similar findings of increased surface hydrophilicity by plasma treatment have been reported for various polymer materials (Fatyeyeva et al., 2014; Meng et al., 2016; Nascimento et al., 2016), as well as plant tissues (Grzegorzewski et al., 2010; Holc et al., 2019; Huang et al., 2019).

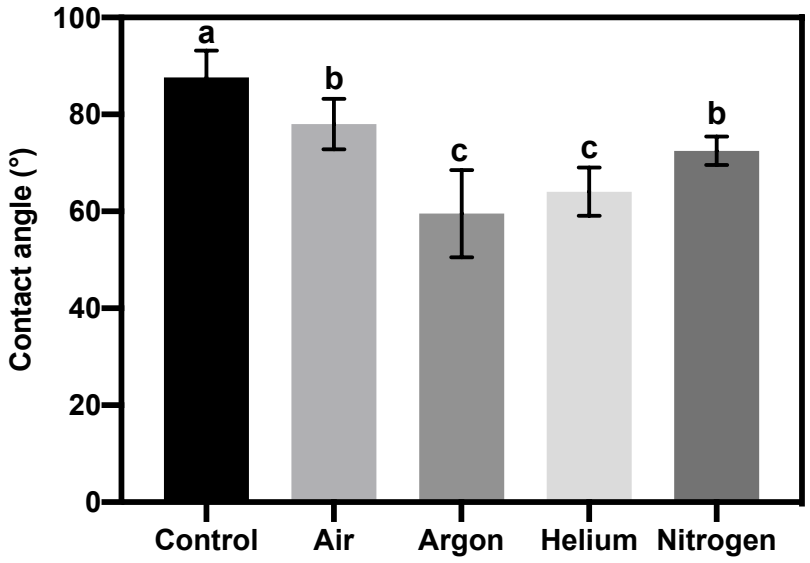

Figure 2.4 Water contact angles of control and HVACP-treated tomato peels. Different letters on the bars represent significant differences among groups ($p < 0.05$).

The increase in surface wettability could be attributed to (i) change in surface chemical composition; (ii) surface energy increase; (iii) degradation of surface hydrophobic layer. It is commonly known that plasma-generated reactive species can modify material surfaces and change their functional groups, in which reactive oxygen and nitrogen species play the most important roles (Fatyeyeva et al., 2014; Holc et al., 2019). Reactive oxygen species can easily convert C=O bond to C-O and C-OH bonds, which are essential chemical bonds responsible for the hydrophilic property (Yang et al., 2005). Reactive nitrogen species can break C-C and/or C-H bonds and activate material surfaces, which can further react with excited nitrogen or oxygen to produce new hydrophilic functional groups, such as carboxyl, carbonate, amine and amide (Fatyeyeva et al., 2014). As mentioned previously, both reactive oxygen and nitrogen species existed in all the plasmas tested in this study due to the air residue, which can increase the O/C and N/C ratios of material surfaces, leading to increased hydrophilicity. Secondly, plasma can

increase the surface energy and roughness of treated surfaces via etching process and hence reduce their hydrophobicity (Meng et al., 2016; Van Deynse et al., 2016). Furthermore, tomato peel is a thin membrane composed of cutin and covered by hydrophobic cuticle waxes (Vogg et al., 2004). Cuticular layer can degrade under exposure to plasma reactive species, resulting in higher surface wettability (Grzegorzewski et al., 2010).

The hydrophobic waxy surface of tomato is highly water-impermeable and can prevent the plant from unregulated water loss (Vogg et al., 2004; Zeisler-Diehl et al., 2018). As described above, plasma reactive species can degrade plant cuticle to make its surface more hydrophilic. This modification can increase the water permeability of plant surfaces and thus water diffusion from the plant interior is expected to be facilitated. Figure 2.5 shows the drying curves of cherry tomato samples at 80 ºC for 12 h. All the HVACP treatments, regardless of the working gas, accelerated the tomato drying. Compared to the control, the most significant enhancement in the moisture loss happened at the ninth hour, of 20.9%, 18.0%, 18.9%, 14.5% for the air, argon, helium and nitrogen plasma-treated samples ($p < 0.05$), respectively. Similarly, Huang et al. (2019) reported an improved drying rate of plasma-treated white grape. The result of enhanced drying after HVACP treatments is consistent with the decreased water contact angle observed, and confirms the capability of HVACP for modifying the hydrophilicity of TP.

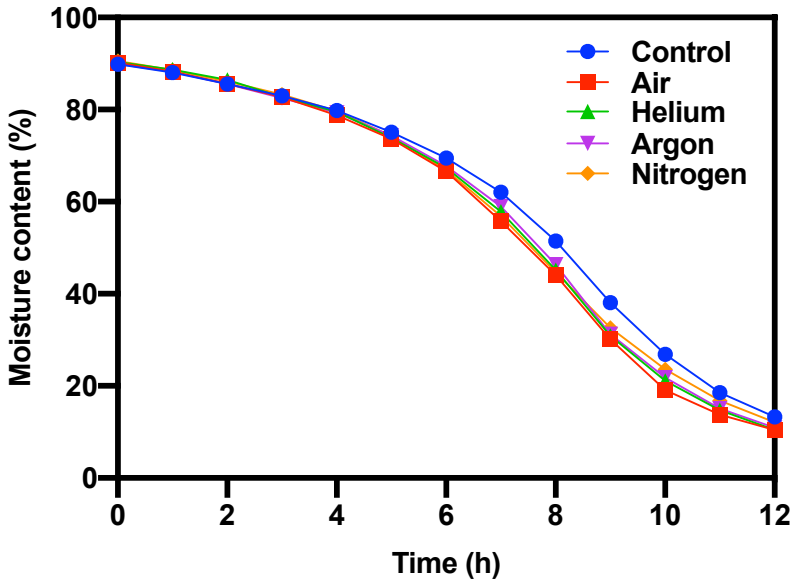

Figure 2.5 Drying curves of control and HVACP-treated cherry tomatoes at 80 ºC. Each point represents the mean value of triplicate tests.

2.3.4 Effect of HVACP treatment on TPC and antioxidant activity of TP extracts

Figure 2.6 shows the TPC of the extracts from different TP samples, expressed based on dry mass. The control had a TPC of 0.941 mg GAE/g. The helium and nitrogen plasma-treated samples both showed higher phenolic extraction yields than the control, by 8.9% and 9.8%, respectively, while air and argon plasma treatments did not exhibit significant improvements. Cold plasma has been used in previous studies to treat various fruits and their products, but different findings regarding TPC were reported. Rodríguez et al. (2017) found that indirect nitrogen-cold plasma treatments for 5−15 min increased the TPC of cashew apple juice. Won et al. (2017) observed an increment of the polyphenol concentration of mandarin peel after 10-min exposure to microwave-powered nitrogen cold plasma at 900 W. In contrast, Ramazzina et al. (2015) reported that DBD cold plasma treatments at 15 kV for 20 min had no effect on the polyphenol content of kiwifruit. Furthermore, the TPC of white grape juice decreased significantly after 1−4 min of exposure to 80-kV HVACP (Pankaj et al., 2017), and similar TPC

reduction was observed in prebiotic orange juice treated by DBD cold plasma at 70 kV for 15−60 s (Almeida et al., 2015).

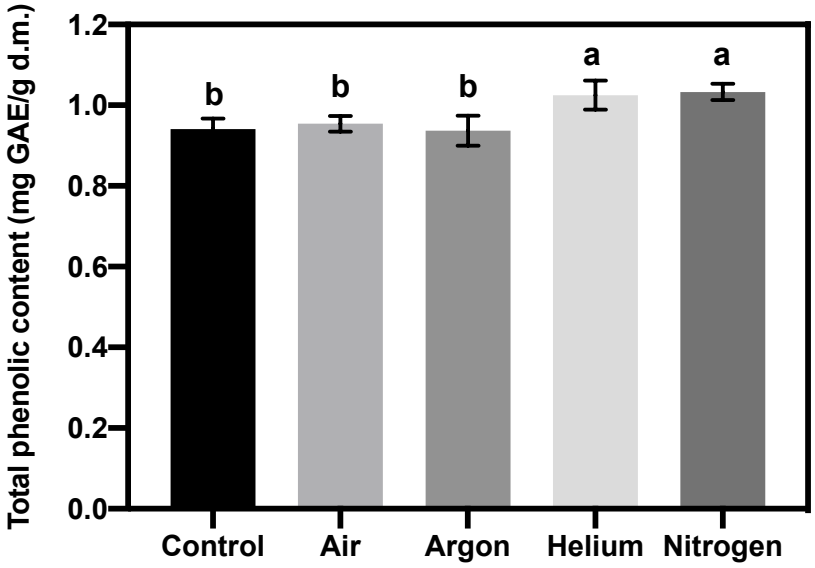

Figure 2.6 Total phenolic content of extracts from control and HVACP-treated tomato pomace at 60 kV for 15 min. Different letters on the bars represent significant differences among groups ($p < 0.05$).

In this study, the enhancement in PC extraction from TP by HVACP treatments can be attributed to three main mechanisms: (i) creation of surface rupture, (ii) increase in surface hydrophilicity, and (iii) cleavage of bond phenolics. As shown in Figures 2.3, the rupture of the TP cell structure can reduce the resistance to molecule movements hence facilitate the release of PC. Meanwhile, the TP with more hydrophilic surfaces favored the diffusion of hydrophilic PC during extraction, resulting in higher extraction efficiency. Plasma treatments can also degrade the outer waxy layer of plants and release a minor fraction of phenolics present in their cutin matrices (Segado et al., 2016). Additionally, some bound phenolics are covalently connected to plant cell wall polysaccharides which only become available when higher energy is applied (Gao et al., 2017; Rodríguez et al., 2017). Reactive gas species generated by HVACP carry sufficient

energy and can induce chemical reactions to break down covalent bonds, hence release the bound phenolics (Ben Belgacem et al., 2017; Çankaya, 2018). Therefore, these three mechanisms associated with HVACP worked synergistically to improve the extraction efficiency of free phenolics and the extractability of bound phenolics.

On the other hand, another effect of HVACP on the TPC of the TP extracts should be noted is that the reactive species and free radicals generated by plasma discharge could induce PC degradation, among which reactive oxygen species and hydroxyl radicals have been widely considered as most impactful (Almeida et al., 2015; Pankaj et al., 2017; Rodríguez et al., 2017). Moreover, the singlet oxygen dissociated from plasma discharge could combine with oxygen molecules to form ozone that can attack the aromatic ring structure of PC and cause their degradation (Misra et al., 2014; Pérez et al., 2002; Stalter et al., 2011). Although the possible PC degradation did not play the dominant role in the HVACP-assisted extraction in this study because of the higher or similar extraction yields obtained (Figure 2.6), it could be a limiting factor for extraction under other operating conditions. Therefore, further studies are needed to optimize the HVACP-assisted extraction, in terms of working gas, treatment time and voltage, which can minimize PC degradation during extraction and thus maximize their extraction yields.

Figure 2.7 shows the antioxidant activity (AA; on the basis of dry mass) of the phenolic extracts from different TP samples. The HVACP treatments, regardless of the working gas, increased the AA of the phenolic extracts. Compared to the control that showed a free radical scavenging activity of 0.684 mg trolox equivalents (TE)/g, the nitrogen plasma-treated sample showed the largest increase, by 30%, which was followed by helium and argon (both by 24%). It is important to note that although the air and argon plasma-treated samples showed similar TPC to the control, their AA increased significantly. The ratios of AA to TPC (i.e., specific

antioxidant activity of phenolics) were 0.727, 0.824, 0.863, 0.864 and 0.902 for the control, and helium, air, nitrogen and argon plasma-treated samples, respectively. The difference in this ratio could be due to the different PC compositions present in the samples, indicating that HVACP treatments have the potential for extracting the PC with higher AA. The AA of tomato peel and seed is mainly related to the hydrophilic phenolics (Toor & Savage, 2005), which became more extractable due to more hydrophilic surfaces formed after HVACP treatments.

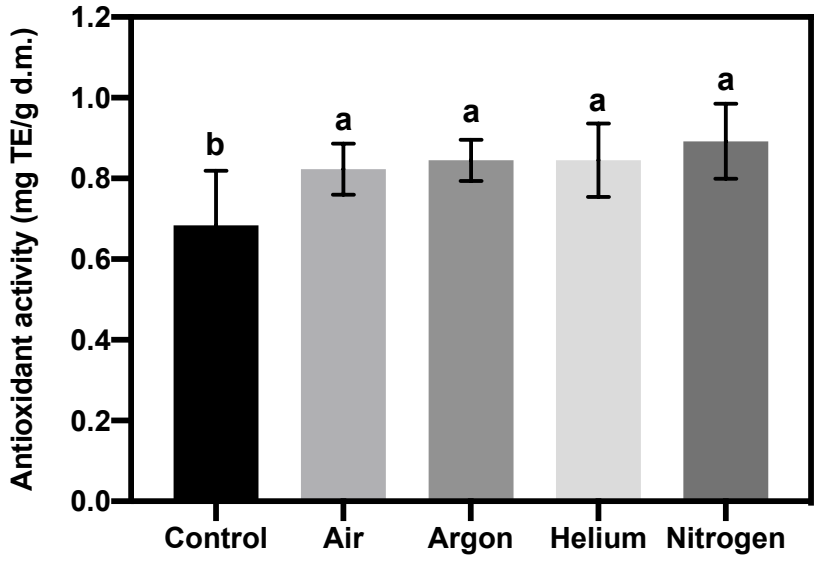

Figure 2.7 Antioxidant activity of extracts from control and HVACP-treated tomato pomace at 60 kV for 15 min. Different letters on the bars represent significant differences among groups ($p < 0.05$).

The AA enhancement by plasma treatments has also been reported by previous studies. Kim et al. (2019) found that low-pressure microwave cold plasma could significantly improve the AA of prickly pear extract. Tappi et al. (2018) reported an increased AA of fresh-cut apple after 10-min DBD plasma treatment because of formation of PC with higher AA.

2.3.5 Effect of HVACP treatment on PC composition

Figures 2.8a and 2.8b present the chromatograms of the phenolic extracts detected at 290 and 350 nm, respectively. Eight PC were identified, including the phenolic acids and flavonoids that are commonly found in tomato (Perea-Domínguez et al., 2018; Valdez-Morales et al., 2014). Chlorogenic acid, caffeic acid, rutin, isoquercetin and quercetin were identified by comparing the retention time and UV spectra of the standards, and trans-ferulic acid was identified by spiking. Gallic acid and naringenin were determined by both methods. These eight PC were also confirmed by the liquid chromatography-mass spectrometry (LC-MS). Table 2.1 summarizes the concentrations of the identified PC in different TP extracts. The concentrations of gallic acid, rutin, isoquercetin and trans-ferulic acid remained unchanged after the HVACP treatments. However, the HVACP treatments, regardless of the working gas, increased the concentrations of caffeic acid, by 25.9% for air and 22.2% for argon, helium and nitrogen. The chlorogenic acid concentration increased by 15.2%, 14.7 % and 18.0% by argon, helium and nitrogen plasmas, respectively, which agreed with Herceg et al. (2016) that argon plasma jet increased the concentration of chlorogenic acid in pomegranate juice. Additionally, both helium and nitrogen plasma treatments significantly increased the concentrations of quercetin and naringenin, which was consistent with the findings that HVACP treatment improved the total flavonols content of grape juice (Pankaj et al., 2017). The extraction of flavonoids was enhanced in a larger extent after HVACP treatments compared to that of phenolic acids, because bound flavonoids can be released with lower energy applied, and plasma etching could potentially facilitate the migration of flavonoids from fruit peel (Rodríguez et al., 2017; Won et al., 2017).

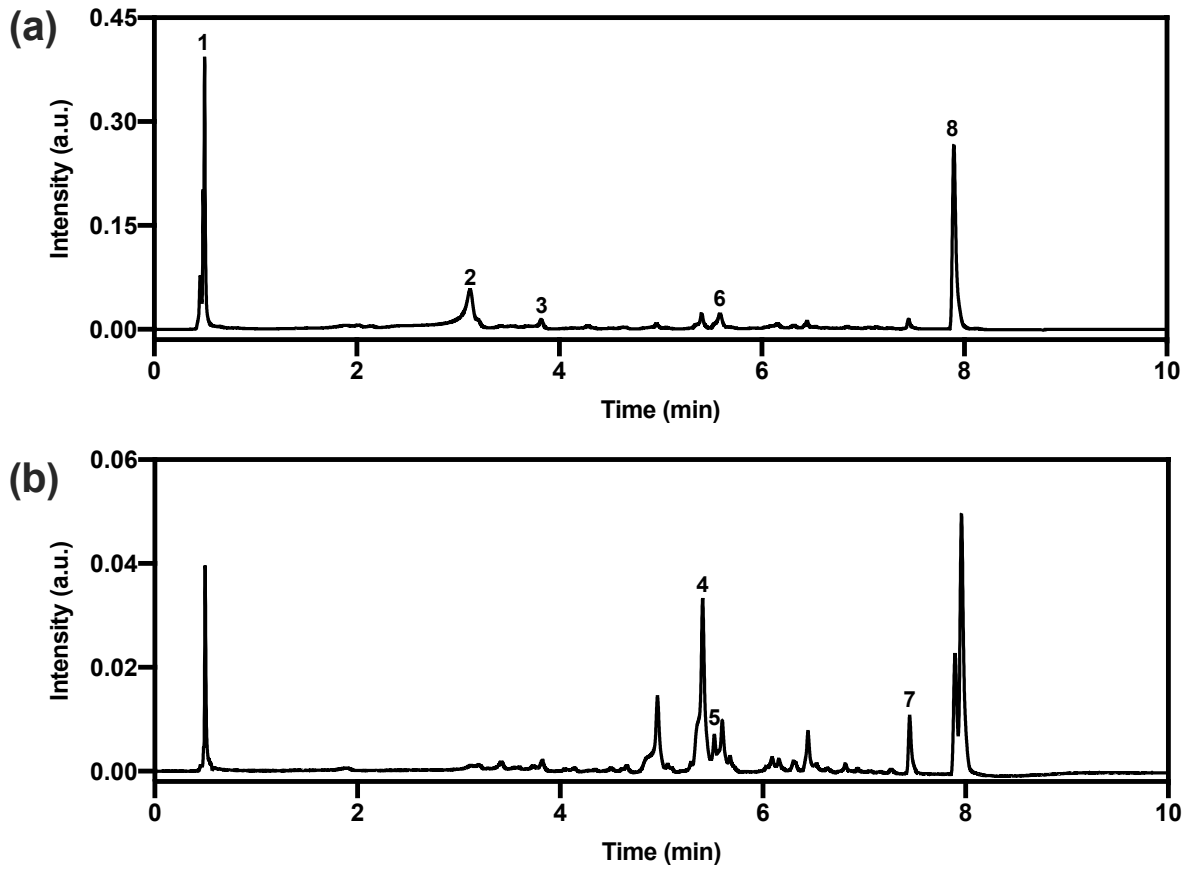

Figure 2.8 UPLC chromatograms of phenolic compounds extracted from tomato pomace detected at (a) 290 nm and (b) 350 nm. Peaks represent (1) gallic acid, (2) chlorogenic acid, (3) caffeic acid, (4) rutin, (5) isoquercetin, (6) trans-ferulic acid, (7) quercetin, (8) naringenin.

Based on the results in Table 2.1, the mechanisms behind the AA increments of air and argon plasma-treated samples are still unclear. One potential reason is that some other phenolic compounds or other bioactive compounds, such as lycopene and ascorbic acid, which we did not measure might have contributed to the increased AA.

Table 2.1 Phenolic compound compositions of extracts from control and HVACP-treated tomato pomace

	Phenolic compounds (mg/g d.w.)							
	Gallic acid	Chlorogenic acid	Caffeic acid	Rutin	Isoquercetin	Trans-ferulic acid	Quercetin	Narigenin
Control	0.861 ± 0.124^{a}	0.645 ± 0.105^{b}	0.027 ± 0.003^{b}	0.180 ± 0.020^{a}	0.025 ± 0.002^{a}	0.072 ± 0.015^{a}	0.030 ± 0.004^{b}	0.424 ± 0.065^{b}
Air	0.787 ± 0.099^{a}	0.716 ± 0.030^{ab}	0.034 ± 0.003^{a}	0.173 ± 0.010^{a}	0.026 ± 0.001^{a}	0.068 ± 0.009^{a}	0.030 ± 0.002^{b}	0.418 ± 0.023^{b}
Argon	0.821 ± 0.072^{a}	0.743 ± 0.015^{a}	0.033 ± 0.001^{a}	0.187 ± 0.006^{a}	0.027 ± 0.001^{a}	0.067 ± 0.005^{a}	0.033 ± 0.002^{ab}	0.431 ± 0.023^{b}
Helium	0.860 ± 0.037^{a}	0.740 ± 0.020^{a}	0.033 ± 0.001^{a}	0.189 ± 0.003^{a}	0.027 ± 0.001^{a}	0.068 ± 0.004^{a}	0.034 ± 0.001^{a}	0.495 ± 0.012^{a}
Nitrogen	0.892 ± 0.026^{a}	0.761 ± 0.024^{a}	0.033 ± 0.002^{a}	0.189 ± 0.006^{a}	0.027 ± 0.001^{a}	0.067 ± 0.007^{a}	0.034 ± 0.001^{a}	0.499 ± 0.006^{a}

All analyses were conducted in triplicate. Data values with different letters in the same column were significantly different ($p < 0.05$).

2.4 Conclusion

This study used HVACP as a pre-treatment to modify TP and investigated its effects on PC extraction. We found that HVACP treatments disrupted the cell wall structure of TP with more hydrophilic surfaces formed, resulting in enhanced PC extraction. Moreover, HVACP treatments enhanced the AA of TP extracts by changing their PC compositions. In conclusion, the developed HVACP-assisted extraction method successfully improved the PC extraction from TP in terms of extraction efficiency and antioxidant capacity of extracts. The findings of this study need to be further explored in order to gain more insights into the mechanisms of plant cell wall rupture caused by cold plasma, and the interactions between PC and plasma reactive species at molecular or atomic level. This novel extraction technology can more effectively valorize tomato processing byproducts with reduced solvent use, hence enhance the sustainability of tomato processing industry. Additionally, it has great potential for recovering other bioactives from different agriculture wastes, which can be utilized as functional food ingredients and nutraceuticals with high nutritional and economic values without causing additional environmental burdens.

Acknowledgements

This work was supported by the USDA National Institute of Food and Agriculture, Hatch project 014964.

References

Almeida, F. D. L., Cavalcante, R. S., Cullen, P. J., Frias, J. M., Bourke, P., Fernandes, F. A. N., & Rodrigues, S. (2015). Effects of atmospheric cold plasma and ozone on prebiotic orange juice. *Innovative Food Science & Emerging Technologies*, *32,* 127–135. https://doi.org/10.1016/j.ifset.2015.09.001

Aspé, E., & Fernández, K. (2011). The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from Pinus radiata Bark. *Industrial Crops and Products,* *34*(1), 838–844. https://doi.org/10.1016/j.indcrop.2011.02.002

Ben Belgacem, Z., Carré, G., Charpentier, E., Le-Bras, F., Maho, T., Robert, E., Pouvesle, J.-M., Polidor, F., Gangloff, S. C., Boudifa, M., & Gelle, M.-P. (2017). Innovative non-thermal plasma disinfection process inside sealed bags: Assessment of bactericidal and sporicidal effectiveness in regard to current sterilization norms. *PLoS ONE,* *12*(6). https://doi.org/10.1371/journal.pone.0180183

Bennamoun, L., Hiligsmann, S., Dakhmouche, S., Ait-Kaki, A., Labbani, F.-Z. K., Nouadri, T., Meraihi, Z., Turchetti, B., Buzzini, P., & Thonart, P. (2016). Production and Properties of a Thermostable, pH-Stable Exo-Polygalacturonase Using Aureobasidium pullulans Isolated from Saharan Soil of Algeria Grown on Tomato Pomace. *Foods (Basel, Switzerland),* *5*(4). https://doi.org/10.3390/foods5040072

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology,* *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Çankaya, N. (2018). *Recent Research in Polymerization.* BoD – Books on Demand.

Cheng, A., Yan, H., Han, C., Chen, X., Wang, W., Xie, C., Qu, J., Gong, Z., & Shi, X. (2014). Acid and Alkaline Hydrolysis Extraction of Non-Extractable Polyphenols in Blueberries: Optimisation by Response Surface Methodology. *Czech J. Food Sci., 32*(3), 8.

Fatyeyeva, K., Dahi, A., Chappey, C., Langevin, D., Valleton, J.-M., Poncin-Epaillard, F., & Marais, S. (2014). Effect of cold plasma treatment on surface properties and gas permeability of polyimide films. *RSC Advances, 4*(59), 31036–31046. https://doi.org/10.1039/C4RA03741C

Gao, Y., Ma, S., Wang, M., & Feng, X.-Y. (2017). Characterization of Free, Conjugated, and Bound Phenolic Acids in Seven Commonly Consumed Vegetables. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry, 22*(11). https://doi.org/10.3390/molecules22111878

Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Phenolic-compound-extraction systems for fruit and vegetable samples. *Molecules (Basel, Switzerland), 15*(12), 8813–8826. https://doi.org/10.3390/molecules15128813

Gilbert, J., Cheng, C. J., & Jones, O. G. (2018). Vapor Barrier Properties and Mechanical Behaviors of Composite Hydroxypropyl Methylcelluose/Zein Nanoparticle Films. *Food Biophysics, 13*(1), 25–36. https://doi.org/10.1007/s11483-017-9508-1

Grzegorzewski, F., Rohn, S., Kroh, L. W., Geyer, M., & Schlüter, O. (2010). Surface morphology and chemical composition of lamb's lettuce (Valerianella locusta) after exposure to a low-pressure oxygen plasma. *Food Chemistry, 122*(4), 1145–1152. https://doi.org/10.1016/j.foodchem.2010.03.104

Han, H., Wang, S., Rakita, M., Wang, Y., Han, Q., & Xu, Q. (2018). Effect of Ultrasound-Assisted Extraction of Phenolic Compounds on the Characteristics of Walnut Shells. *Food and Nutrition Sciences, 9*(8), 1034–1045. https://doi.org/10.4236/fns.2018.98076

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P. J., & Bourke, P. (2016). Mechanisms of Inactivation by High-Voltage Atmospheric Cold Plasma Differ for Escherichia coli and Staphylococcus aureus. *Applied and Environmental Microbiology, 82*(2), 450–458. https://doi.org/10.1128/AEM.02660-15

Herceg, Z., Kovačević, D. B., Kljusurić, J. G., Jambrak, A. R., Zorić, Z., & Dragović-Uzelac, V. (2016). Gas phase plasma impact on phenolic compounds in pomegranate juice. *Food Chemistry, 190*, 665–672. https://doi.org/10.1016/j.foodchem.2015.05.135

Hoentsch, M., Bussiahn, R., Rebl, H., Bergemann, C., Eggert, M., Frank, M., von Woedtke, T., & Nebe, B. (2014). Persistent Effectivity of Gas Plasma-Treated, Long Time-Stored Liquid on Epithelial Cell Adhesion Capacity and Membrane Morphology. *PLoS ONE, 9*(8). https://doi.org/10.1371/journal.pone.0104559

Hoffmann, C., Berganza, C., & Zhang, J. (2013). Cold Atmospheric Plasma: Methods of production and application in dentistry and oncology. *Medical Gas Research, 3*, 21. https://doi.org/10.1186/2045-9912-3-21

Holc, M., Primc, G., Iskra, J., Titan, P., Kovač, J., Mozetič, M., & Junkar, I. (2019). Effect of Oxygen Plasma on Sprout and Root Growth, Surface Morphology and Yield of Garlic. *Plants, 8*(11), 462. https://doi.org/10.3390/plants8110462

Huang, C.C., Wu, J. S.B., Wu, J.S., & Ting, Y. (2019). Effect of novel atmospheric-pressure jet pretreatment on the drying kinetics and quality of white grapes. *Journal of the Science of Food and Agriculture, 99*(11), 5102–5111. https://doi.org/10.1002/jsfa.9754

Joshi, S. G., Cooper, M., Yost, A., Paff, M., Ercan, U. K., Fridman, G., Friedman, G., Fridman, A., & Brooks, A. D. (2011). Nonthermal Dielectric-Barrier Discharge Plasma-Induced Inactivation Involves Oxidative DNA Damage and Membrane Lipid Peroxidation in Escherichia coli. *Antimicrobial Agents and Chemotherapy, 55*(3), 1053–1062. https://doi.org/10.1128/AAC.01002-10

Kelley, W. T., Boyhan, G. E., Harrison, K. A., Sumner, P. E., Langston, D. B., Sparks, A. N., Culpepper, S., Hurst, W. C., & Fonsah, E. G. (2010). *Commercial tomato production handbook.*

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules (Basel, Switzerland), 18*(2), 2328–2375. https://doi.org/10.3390/molecules18022328

Kim, S. Y., Lee, S. Y., & Min, S. C. (2019). Improvement of the Antioxidant Activity, Water Solubility, and Dispersion Stability of Prickly Pear Cactus Fruit Extracts Using Argon Cold Plasma Treatment. *Journal of Food Science, 84*(10), 2876–2882. https://doi.org/10.1111/1750-3841.14791

Knoblich, M., Anderson, B., & Latshaw, D. (2005). Analyses of tomato peel and seed byproducts and their use as a source of carotenoids. *Journal of the Science of Food and Agriculture, 85*(7), 1166–1170. https://doi.org/10.1002/jsfa.2091

Kodama, S., Thawatchaipracha, B., & Sekiguchi, H. (2014). Enhancement of Essential Oil Extraction for Steam Distillation by DBD Surface Treatment. *Plasma Processes and Polymers, 11*(2), 126–132. https://doi.org/10.1002/ppap.201300047

Li, M., Koecher, K., Hansen, L., & Ferruzzi, M. G. (2016). Phenolic recovery and bioaccessibility from milled and finished whole grain oat products. *Food & Function, 7*(8), 3370–3381. https://doi.org/10.1039/C6FO00760K

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules, 21*(10). https://doi.org/10.3390/molecules21101374

Lotfy, K., Khalil, S. M., & El-Raheem, H. A. (2020). Inactivation by helium cold atmospheric pressure plasma for Escherichia coli and Staphylococcus aureus. *Journal of Theoretical and Applied Physics, 14*(1), 37–45. https://doi.org/10.1007/s40094-019-00362-4

Madhujith, T., & Shahidi, F. (2009). Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chemistry, 117*(4), 615–620. https://doi.org/10.1016/j.foodchem.2009.04.055

Meng, J., Wang, Y., Wang, Y., & Ding, Z.-Q. (2016). Effect of cold atmospheric plasma treatment on hydrophilic properties of fluorosilicone rubber. *Surface and Interface Analysis, 48*(13), 1429–1435. https://doi.org/10.1002/sia.6053

Misra, N. N., Patil, S., Moiseev, T., Bourke, P., Mosnier, J. P., Keener, K. M., & Cullen, P. J. (2014). In-package atmospheric pressure cold plasma treatment of strawberries. *Journal of Food Engineering, 125*, 131–138. https://doi.org/10.1016/j.jfoodeng.2013.10.023

Nascimento, F. do, Moshkalev, S., Machida, M., & Parada, S. (2016). Treatment of PDMS surfaces using pulsed DBD plasmas: Comparing the use of different gases and its influence on adhesion. *Japanese Journal of Applied Physics, 55*(2), 021602. https://doi.org/10.7567/JJAP.55.021602

Pankaj, S. K., Wan, Z., Colonna, W., & Keener, K. M. (2017). Effect of high voltage atmospheric cold plasma on white grape juice quality. *Journal of the Science of Food and Agriculture, 97*(12), 4016–4021. https://doi.org/10.1002/jsfa.8268

Perea-Domínguez, X. P., Hernández-Gastelum, L. Z., Olivas-Olguin, H. R., Espinosa-Alonso, L. G., Valdez-Morales, M., & Medina-Godoy, S. (2018). Phenolic composition of tomato varieties and an industrial tomato by-product: Free, conjugated and bound phenolics and antioxidant activity. *Journal of Food Science and Technology, 55*(9), 3453–3461. https://doi.org/10.1007/s13197-018-3269-9

Pérez, M., Torrades, F., Domènech, X., & Peral, J. (2002). Treatment of bleaching Kraft mill effluents and polychlorinated phenolic compounds with ozonation. *Journal of Chemical Technology & Biotechnology, 77*(8), 891–897. https://doi.org/10.1002/jctb.651

Poletti, G., Orsini, F., Raffaele-Addamo, A., Riccardi, C., & Selli, E. (2003). Cold plasma treatment of PET fabrics: AFM surface morphology characterisation. *Applied Surface Science, 219*(3), 311–316. https://doi.org/10.1016/S0169-4332(03)00703-7

Ramazzina, I., Berardinelli, A., Rizzi, F., Tappi, S., Ragni, L., Sacchetti, G., & Rocculi, P. (2015). Effect of cold plasma treatment on physico-chemical parameters and antioxidant activity of minimally processed kiwifruit. *Postharvest Biology and Technology, 107*, 55–65. https://doi.org/10.1016/j.postharvbio.2015.04.008

Rodríguez, Ó., Gomes, W. F., Rodrigues, S., & Fernandes, F. A. N. (2017). Effect of indirect cold plasma treatment on cashew apple juice (Anacardium occidentale L.). *LWT, 84*, 457–463. https://doi.org/10.1016/j.lwt.2017.06.010

Segado, P., Domínguez, E., & Heredia, A. (2016). Ultrastructure of the Epidermal Cell Wall and Cuticle of Tomato Fruit (Solanum lycopersicum L.) during Development1[OPEN]. *Plant Physiology, 170*(2), 935–946. https://doi.org/10.1104/pp.15.01725

Seol, Y., Kim, J., Park, S., & Young Chang, H. (2017). Atmospheric Pressure Pulsed Plasma Induces Cell Death in Photosynthetic Organs via Intracellularly Generated ROS. *Scientific Reports, 7*. https://doi.org/10.1038/s41598-017-00480-6

Shi, H., Ileleji, K., Stroshine, R. L., Keener, K., & Jensen, J. L. (2017). Reduction of Aflatoxin in Corn by High Voltage Atmospheric Cold Plasma. *Food and Bioprocess Technology, 10*(6), 1042–1052. https://doi.org/10.1007/s11947-017-1873-8

Slinkard, K., & Singleton, V. L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American Journal of Enology and Viticulture, 28*(1), 49–55.

Stalter, D., Magdeburg, A., Wagner, M., & Oehlmann, J. (2011). Ozonation and activated carbon treatment of sewage effluents: Removal of endocrine activity and cytotoxicity. *Water Research, 45*(3), 1015–1024. https://doi.org/10.1016/j.watres.2010.10.008

Su, D., Zhang, R., Hou, F., Zhang, M., Guo, J., Huang, F., Deng, Y., & Wei, Z. (2014). Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solvents. *BMC Complementary and Alternative Medicine, 14,* 9. https://doi.org/10.1186/1472-6882-14-9

Suwal, S., Coronel-Aguilera, C. P., Auer, J., Applegate, B., Garner, A. L., & Huang, J.Y. (2019). Mechanism characterization of bacterial inactivation of atmospheric air plasma gas and activated water using bioluminescence technology. *Innovative Food Science & Emerging Technologies, 53*, 18–25. https://doi.org/10.1016/j.ifset.2018.01.007

Tappi, S., Ramazzina, I., Rizzi, F., Sacchetti, G., Ragni, L., & Rocculi, P. (2018). Effect of Plasma Exposure Time on the Polyphenolic Profile and Antioxidant Activity of Fresh-Cut Apples. *Applied Sciences, 8*(10), 1939. https://doi.org/10.3390/app8101939

Toor, R. K., & Savage, G. P. (2005). Antioxidant activity in different fractions of tomatoes. *Food Research International, 38*(5), 487–494. https://doi.org/10.1016/j.foodres.2004.10.016

Valdez-Morales, M., Espinosa-Alonso, L. G., Espinoza-Torres, L. C., Delgado-Vargas, F., & Medina-Godoy, S. (2014). Phenolic content and antioxidant and antimutagenic activities in tomato peel, seeds, and byproducts. *Journal of Agricultural and Food Chemistry, 62*(23), 5281–5289. https://doi.org/10.1021/jf5012374

Van Deynse, A., Morent, R., & De Geyter, N. (2016). Surface modification of polymers using atmospheric pressure cold plasma technology. In Polymer science: Research advances, pratical applications and educational aspects (pp. 506–516). *Formatex Research Center.* http://hdl.handle.net/1854/LU-8088361

Ventura, M. R., Pieltain, M. C., & Castanon, J. I. R. (2009). Evaluation of tomato crop by-products as feed for goats. https://pubag.nal.usda.gov/catalog/769136

Vogg, G., Fischer, S., Leide, J., Emmanuel, E., Jetter, R., Levy, A. A., & Riederer, M. (2004). Tomato fruit cuticular waxes and their effects on transpiration barrier properties: Functional characterization of a mutant deficient in a very-long-chain fatty acid β-ketoacyl-CoA synthase. *Journal of Experimental Botany, 55*(401), 1401–1410. https://doi.org/10.1093/jxb/erh149

Won, M. Y., Lee, S. J., & Min, S. C. (2017). Mandarin preservation by microwave-powered cold plasma treatment. *Innovative Food Science & Emerging Technologies, 39*, 25–32. https://doi.org/10.1016/j.ifset.2016.10.021

Xu, L., Garner, A. L., Tao, B., & Keener, K. M. (2017). Microbial Inactivation and Quality Changes in Orange Juice Treated by High Voltage Atmospheric Cold Plasma. *Food and Bioprocess Technology, 10*(10), 1778–1791. https://doi.org/10.1007/s11947-017-1947-7

Yang, C., Lee, S., Wu, J., & Lin, T. (2005). The properties of copper films deposited on polyimide by nitrogen and oxygen plasma pre-treatment. *Applied Surface Science, 252*(5), 1818–1825. https://doi.org/10.1016/j.apsusc.2005.03.182

Zeisler-Diehl, V. V., Barthlott, W., & Schreiber, L. (2018). Plant Cuticular Waxes: Composition, Function, and Interactions with Microorganisms. In H. Wilkes (Ed.), *Hydrocarbons, Oils and Lipids: Diversity, Origin, Chemistry and Fate* (pp. 1–16). Springer International Publishing. https://doi.org/10.1007/978-3-319-54529-5_7-1

Zhang, H., Xu, Z., Shen, J., Li, X., Ding, L., Ma, J., Lan, Y., Xia, W., Cheng, C., Sun, Q., Zhang, Z., & Chu, P. K. (2015). Effects and Mechanism of Atmospheric-Pressure Dielectric Barrier Discharge Cold Plasmaon Lactate Dehydrogenase (LDH) Enzyme. *Scientific Reports, 5*(1), 1–12. https://doi.org/10.1038/srep10031

CHAPTER 3. ENHANCEMENT OF PHENOLIC COMPOUNDS EXTRACTION FROM GRAPE POMACE BY HIGH VOLTAGE ATMOSPHERIC COLD PLASMA

Yiwen Bao[a], Lavanya Reddivari[a], Jen-Yi Huang[a,b,*]

[a]Department of Food Science, Purdue University, West Lafayette, IN, USA

[b]Environmental and Ecological Engineering, Purdue University, West Lafayette, IN, USA

*Corresponding author. Tel.: +1-765-496-6034, Fax: +1-765-494-7953

Address: 745 Agriculture Mall Drive, West Lafayette, IN 47907, USA

E-mail: huang874@purdue.edu

Submitted to

Journal of Food Engineering

April 2020

Abstract

The huge amount of grape pomace (GP) generated along with wine production usually ends up with waste or uses with low economic value. However, due to the high content of phenolic compounds (PC), GP can be valorized through extraction. As a partially ionized gas, cold plasma carries various reactive species which have proved effective in modifying material surfaces. This study applied high voltage (60 kV) atmospheric cold plasma (HVACP) as a pretreatment on GP for different time periods (5, 10 and 15 min) to assist in its PC extraction, in terms of extraction yields, and the composition and antioxidant activity (AA) of GP extracts. HVACP treatment was found to disrupt the epidermal cell structures of GP, reduce the water contact angle of grape peels, as well as accelerate grape drying, and the effects became more significant as treatment extended. HVACP treatment also increased the yield of phenolic extracts, by 10.9−22.8%, which contained a higher concentration of anthocyanin and showed an improved AA (16.7−34.7%). These results demonstrate that the developed HVACP-assisted extraction process can successfully advance the extraction of bioactive compounds by not only increase their yield but also improve their nutritional quality. This is a promising technology for winemaking industry to valorize grape processing byproducts for functional food and nutraceutical applications.

Keywords: *Cold plasma-assisted extraction; Grape processing byproducts; Surface modification; Bioactive compounds; Anthocyanin; Antioxidant activity*

Highlights

- A novel cold plasma-assisted extraction technology is developed for PC from GP
- Cold plasma causes structural damage to GP cell wall
- Cold plasma increases grape peel hydrophilicity and drying rate of tomato grape
- Treating GP with cold plasma increases its PC extraction yield.
- Extracts from cold plasma-treated GP have higher AA and different PC composition

3.1 Introduction

Grape is the most popular fruit crops in the world, of which about 80% are used for winemaking (Pinelo et al., 2006). Grape pomace (GP) is a processing residue from wine or juice production and weighs 20% of harvested grape (Tseng & Zhao, 2012). The huge amount of GP generated is commonly used as a byproduct for animal feeds or directly distributed to landfill as waste, ending up with low values and heavy environmental burdens. However, due to incomplete extraction during wine production, GP contains a high concentration of phenolic compounds (PC) and thus has great potential for valorization through PC recovery (Rockenbach et al., 2011). The polyphenols in GP have a wide variety of antioxidant components, including anthocyanins, flavonols, flavanols, phenolic acids and stilbenes. These phenolics have many benefits to human health, such as preventing heart disease, inhibiting macromolecular oxidation, anti-inflammatory and anti-carcinogenic properties. (Lin et al., 2016; Negro et al., 2003). Moreover, anthocyanin is considered as an alternative natural colorant in food industry because it is from plant sources with bright color and high water solubility (Rockenbach et al., 2011). Therefore, extracting PC from GP for value-added products has attracted growing attention.

In GP, PC can be in either free form or bound form. The extraction of bound PC is difficult since they are hindered by the cell wall structure of GP which may even entrap free PC as well. Therefore, the choice of extraction method can greatly influence the PC extraction yield (Khoddami et al., 2013). The conventional methods for PC extraction include liquid-liquid and solid-liquid extractions based on organic solvents such as methanol, ethanol, acetone, etc. (Faller & Fialho, 2009). However, these organic solvents not only have relatively low efficiency in extraction, but also induce food safety concerns as well as chemical pollution to the environment (Aspé & Fernández, 2011). Besides, the absence of standardized PC extraction protocols prevents their wide nutraceutical uses (Rousseaux & Schachter, 2003). Thus, an efficient and green extraction method to increase PC extractability while reducing chemical solvent usage is needed.

Plasma is a partially ionized gas containing free electrons, ions, radicals, excited and non-excited molecules and photons (Pankaj & Keener, 2017). Cold plasma, also known as nonthermal plasma, is operated at a temperature lower than 40 ºC under atmospheric pressure (Hoffmann et al., 2013). Cold plasma is widely used for polymer modification and functionalization because it has proven abilities to modulate surface wettability and roughness (Fatyeyeva et al., 2014; Van Deynse et al., 2016). Cold plasma has also been used in the biomedical fields for wound healing, skin dentistry, cancer treatment, drug delivery, etc. (Fridman et al., 2008; Keidar et al., 2011; Shashurin et al., 2008). Owing to the nonthermal and energy-efficient features, cold plasma has emerged in recent years as a promising technology for food processing. Cold plasma has great microbial inactivation efficacy due to the strong oxidative stress and cell membrane leakage caused by plasma reactive species to target microorganisms (Han et al., 2016; Xu et al., 2017). Cold plasma has also been used to modify the

quality of food products, including availability and antioxidant capacity of bioactive compounds. Bursać Kovačević et al. (2016) found the total anthocyanin content in pomegranate juice increased by 21−35% after exposure to plasma, and Won et al. (2017) indicated that plasma treatments increased the antioxidant activity of mandarin peel. Other food-related applications include surface wettability modification, cell structure disruption, and nutritional value enhancement (Grzegorzewski et al., 2010; Seol et al., 2017; Sainz-García et al., 2019).

The unique characteristics of cold plasma described above highlight its potential for agricultural byproducts treatments in order to assist in extraction of their bioactive compounds. However, the effect of cold plasma treatment on GP properties and the extraction of its PC has not been studied. Therefore, this study aimed to develop a novel cold plasma-assisted extraction process to increase the extraction efficiency of PC, especially anthocyanin, from GP.

3.2 Material and methods

3.2.1 Materials

Winemaking byproducts from Cabernet Sauvignon (*Vitis vinifera*) were provided by the Oregon State University Research Winery (Corvallis, OR, USA). Grape stems were manually removed from byproducts to obtain GP. Black seedless grapes were purchased from the Walmart Supercenter (West Lafayette, IN, USA) to study the effects of cold plasma on the surface properties of grape peel and grape drying kinetics.

Reagents used for extraction and chemical assays (total phenolic content, total anthocyanin content, and antioxidant activity) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Analytical standards of gallic acid, protocatechuic acid, quercetin, malvidin, cyanidin, delphinidin, petunidin, peonidin and 7-hydroxycoumarin were purchased from Sigma-Aldrich

(St. Louis, MO, USA). Solvents for ultra-performance liquid chromatography (UPLC) analysis were purchased from VWR International (Bristol, CT, USA).

3.2.2 Sample preparation

GP was freeze-dried for 48 h before ground into powder (< 0.841 mm) then stored under −20 ºC for further use. All the samples were pre-packed for plasma treatment. GP powder (0.5 g) was evenly dispersed in a glass crystallizing dish (8-cm diameter) sealed with one layer of Parafilm™ to limit powder movement caused by the strong electric field within the petri dish. The container was then filled with helium gas and sealed again using Parafilm™. For contact angle and drying kinetics characterization, a piece of grape peel (1 × 1 cm) cut from black seedless grape or a whole grape fruit was placed in a polypropylene box (27.9 × 18.4 × 4.4 cm), which was flushed with helium and sealed using a high barrier film (B2630T, Cryovac® Sealed Air Inc, NJ, USA).

3.2.3 High voltage atmospheric cold plasma treatment

In this study, high voltage atmospheric cold plasma (HVACP) was applied on the samples, as shown in Figure 3.1. The plasma was generated by dielectric barrier discharge (DBD) using a Phenix BK-130 transformer (Phenix Technologies, MD, USA) operated at the input voltage of 120 V (AC) at 60 Hz (Suwal et al., 2019; Xu et al., 2017). The sealed crystallizing dish/package containing the test sample was placed between polypropylene pads (355 × 272 × 2.20 mm, Cuisinart, NJ, USA) as dielectric barriers. Two 15.24-cm-diameter circular aluminum electrodes were respectively attached to the top and bottom dielectric barriers, creating a fixed gap of 5.2

cm for plasma generation. Samples were exposed under 60-kV cold plasma for 5, 10 and 15 min and kept for further analyses.

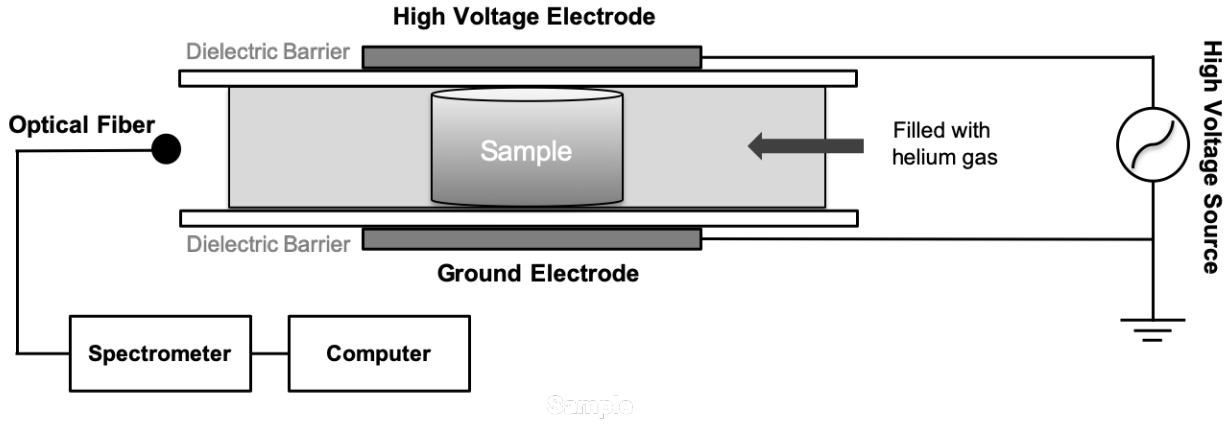

Figure 3.1 Schematic diagram of the experimental setup for high voltage atmospheric cold plasma (HVACP) treatment and optical emission spectroscopy (OES) analysis.

3.2.4 Optical emission spectroscopy

The reactive species generated during HVACP treatment were monitored using optical emission spectroscopy (OES). The spectrum was recorded by a HR2000+ spectrometer (Ocean Optics, Largo, FL, USA). The emission light was captured using a collimating lens (UV-VIS, 200-2000 nm) and delivered by an optical fiber with a core size of 400 μm. The distance between the lens and the plasma chamber was 140 mm. Data was recorded every 30 s using the OceanView software and corrected with the background noise.

3.2.5 Scanning electron microscopy (SEM)

The surface microstructure of GP was visualized by scanning electron microscopy (SEM; FEI Quanta 3D FEG, FEI Company, Hillsboro, OR, USA) equipped with an Everhart-Thornley Detector (ETD). GP powder samples were fixed on SEM discs by double-sided carbon tape and

sputter-coated with platinum for 60 s. The SEM images were captured at an accelerating voltage of 5 kV within a working range between 10.8 and 11.4 mm.

3.2.6 Water contact angle measurements

The water contact angle of grape peel was measured using an Attension Theta Tensiometer (Biolin Scientific, Paramus, NJ, USA) to determine the effect of HVACP treatment on its surface hydrophilicity. The measurements were conducted at room temperature with 2 μL of water droplet placed on the surface of grape peels. The values of the right and left contact angles were averaged and recorded by the One Attention software.

3.2.7 Phenolic compounds extraction

PC were extracted from GP according to the procedure developed by Librán et al. (2013) with modifications. GP (0.4 g) was well mixed with 10 mL of 50% (v/v) ethanol then stirred continuously using an incubator shaker at 150 rpm. After incubation at room temperature for 2 h, the mixture was centrifuged at 2000 × g for 15 min. The supernatant was collected and stored at −80 ºC.

3.2.8 Total phenolic content and total anthocyanin content

The total phenolic content (TPC) of the GP extract was measured according to the Folin-Ciocalteu micro-method (Slinkard & Singleton, 1977). GP extract (35 μL) was mixed with 150 μL of Folin-Ciocalteu's reagent (diluted by ten times) then continuously shaken at 400 rpm for 30 s. After 5-min incubation at room temperature, Na_2CO_3 solution (7.5%, w/v; 115 μL) was added into the mixture with another 30 s of shaking at 400 rpm before incubated at 45 ºC for 30 min then at room temperature for additional 1 h. The TPC was determined by the optical

absorbance of the final mixture measured by a Cytation 1 multi-mode reader (BioTek Instruments Inc., Winooski, VT, USA) at 765 nm wavelength. A standard curve of gallic acid was prepared with gradient concentrations from 40 to 200 μg/mL.

The total anthocyanin content (TAC) of the GP extract was determined by the pH differential method (Giusti & Wrolstad, 2001). Sixty microliters of the extract were diluted with both 240 μL of potassium chloride buffer (pH 1.0; 0.025 M) and 240 μL of sodium acetate buffer (pH 4.5; 0.4 M). The mixture was shaken at 400 rpm for 30 s and equilibrated for 15 min. The readings were recorded at both 520 and 700 nm. The TAC (mg C_3G/mL) was calculated by:

$$\mathrm{TAC} = [(A_{520}, pH\ 1 - A_{700}, pH\ 1) - (A_{520}, pH\ 4.5 - A_{700}, pH\ 4.5)] \times 449.2 \times \frac{DF}{26900} \quad \text{Eq. (1)}$$

where A refers to the absorbance measured at different pH (1 and 4.5) and wavelengths (520 and 700 nm), the constants 449.2 (g/mol) and 26900 (L/cm/mol) are the molar mass and molar absorptivity of cyanidin-3-glucoside, and DF is the dilution factor of GP extract which is 5 here (60 μL extract to 240 μL buffer).

3.2.9 Antioxidant activity

The antioxidant activity (AA) of the GP extract was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity analysis (Brand-Williams et al., 1995). Fifteen microliters of extract were added into 285 μL of DPPH solution under shaking (400 rpm, 30 s). The mixture was incubated in dark at room temperature for 2 h. Decolorization of the solution was measured by optical absorbance at 515 nm. A standard curve of trolox was prepared with a concentration gradient from 40 to 200 μg/mL.

3.2.10 Acid hydrolysis of GP extracts

Acid hydrolysis was performed to convert the anthocyanins in GP extracts to anthocyanidins following the method described by Truong et al. (2010). The internal standard (7-hydroxycoumarin) was mixed with 6 N HCl for a final concentration of 20 μM. GP extract (300 μL) was added into 450 μL of HCl in a 9-mL glass tube with screw cap. The mixture was hydrolyzed at 100 ºC for 30 min then immediately cooled in an ice bath. The hydrolyzed extract was dried using a Savant™ SpeedVac (Thermo Fisher Scientific, Waltham, MA, USA) for 3 h then redissolved in 300 μL of 50 % ethanol with 0.2 % formic acid.

3.2.11 UPLC analysis

The characterization and quantification of the PC in GP extracts followed the procedure developed by Li et al. (2016) with minor modifications using a Waters Acquity H-Class UPLC system (Milford, MA, USA) coupled with a photodiode array detector (PAD). A gradient elution system was carried out to separate individual compound on a Waters BEH C18 column (2.1 × 100 mm, and 1.7 μm) at 40 ºC with the elution solvents of A: 0.2% formic acid in acetonitrile (ACN), and B: 0.2% formic acid in water/ACN (95/5). The flow rate was set at 0.5 mL/min and the sample injection volume was 10 μL. The gradient program followed: 0% A/100% B at 0 min, 9% A/91% B at 3 min, 32% A/68% B at 7 min, and back to the initial condition of 100% B at 8 min. Eluates were monitored at 290, 350 and 520 nm. PC were identified by comparing retention times and UV spectra with the standards, and quantified using the calibration curves for gallic acid, protocatechuic acid, quercetin, and the chloride form of anthocyanidin (delphinidin, cyanidin, peonidin, petunidin and malvidin).

3.2.12 Statistical analysis

All experiments were conducted in triplicate, and data is presented as mean ± standard deviation. Data was analyzed by one-way analysis of variance (ANOVA) using SPSS v23.0 (SPSS Inc., Chicago, IL, USA) followed by Tukey's multiple comparison test ($p < 0.05$) to assess group differences.

3.3 Results and discussion

3.3.1 Plasma reactive species in HVACP

OES was used to characterize the reactive species generated by DBD plasma. The emission spectrum of the HVACP with helium at 60 kV between 200 and 1000 nm is displayed in Figure 3.2. Major emission peaks within the wavelength region of 587−728 nm indicated the existence of helium metastable atoms, and the peak at 389 nm also represented the helium atom (Zhang et al., 2015). However, it is important to note that the spectrum of the range between 315 and 427 nm was dominated by the second positive system of N_2 and the first negative system of N_2^+ (Xu et al., 2017). Moreover, exited atomic oxygen species was observed at 777 nm (Pankaj et al., 2017). The generation of N_2 and N_2^+ species is due to the penning effect of metastable He, and O species usually comes from disassociating oxygen and water molecules in the air (Wang et al., 2016). Their presence suggests that there was air residue remained in the plasma chamber which was also ionized to produce reactive oxygen and nitrogen species that may participate in the treatments on samples. The helium-plasma spectrum monitored in this study is similar to those reported in previous studies (Nastuta et al., 2011; Zhang et al., 2015).

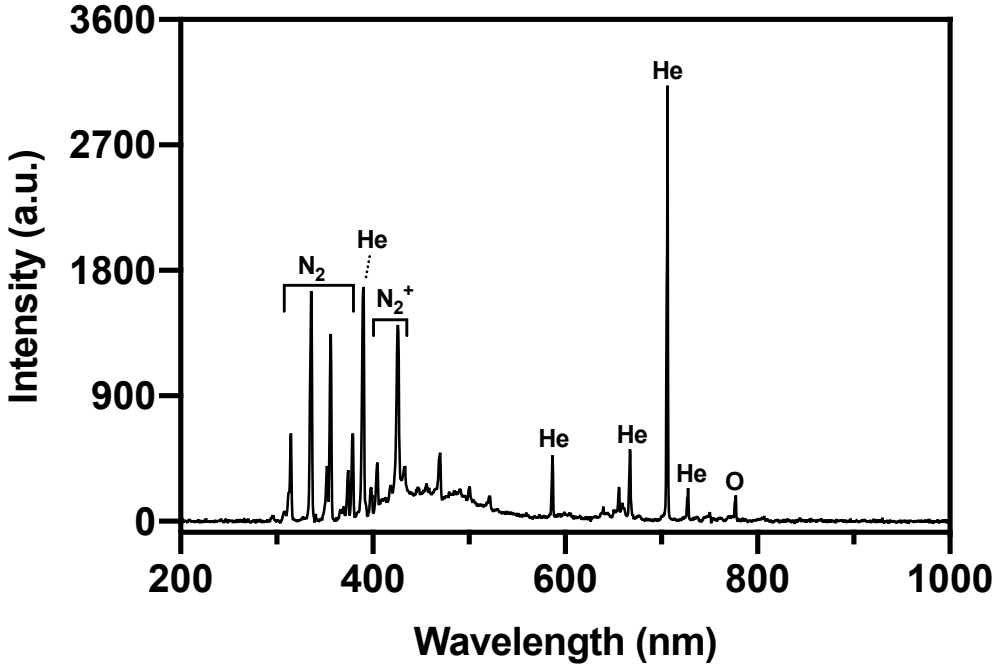

Figure 3.2 Optical emission spectra of HVACP generated with helium gas at 60 kV.

3.3.2 Surface microstructure of grape pomace

SEM imaging was used to visualize the effect of HVACP treatment on the surface microstructure of GP, as shown in Figure 3.3. The untreated GP (control; Figure 3.3a) displayed explicitly defined contours of the epidermal cells with thick cell wall layer, which is similar to the grape skin surface microstructure reported by Huang et al. (2005). The rectangle-shaped cells appeared to be less visible after 5- (Figure 3.3b) and 10-min (Figure 3.3c) HVACP treatments because the outer cell wall became thinner and some ruptured fragments were observed. Greater changes in the surface morphology can be seen when the treatment continued to 15 min (Figure 3d), the GP had no visible cell wall structure and the whole cell completely lost its integrity. Similar findings were also reported by Seol et al. (2017) that needle-shaped plasma jet operated with helium induced direct damage on cell wall structure in plant epidermis tissue. In addition to

cell wall structure disruption, plasma was found to cause other types of surface morphology change in plant tissue, including roughing lettuce surface (Grzegorzewski et al., 2010), creating cracks on white grape skin (Huang et al., 2019), and scorching lemon peel surface (Kodama et al., 2014). Pan et al. (2020) also reported that prolonging DBD plasma treatment from 2 to 4 min caused more profound shrinkage and deformation on *L. monocytogenes* cells. Furthermore, the rupture of bacterial cells induced by accumulated plasma reactive species has been widely studied. Xu et al. (2017) found *S. enterica* cells lost their integrity after 120 s of HVACP treatment at 90 kV due to the etching effect, resulting in irregular surfaces. Nishime et al. (2017) indicated that plasma jet at 13 kV for 120 s induced surface morphology changes and intracellular material losses of *C. albicans, E. faecalis* and *P. aeruginosa* cells.

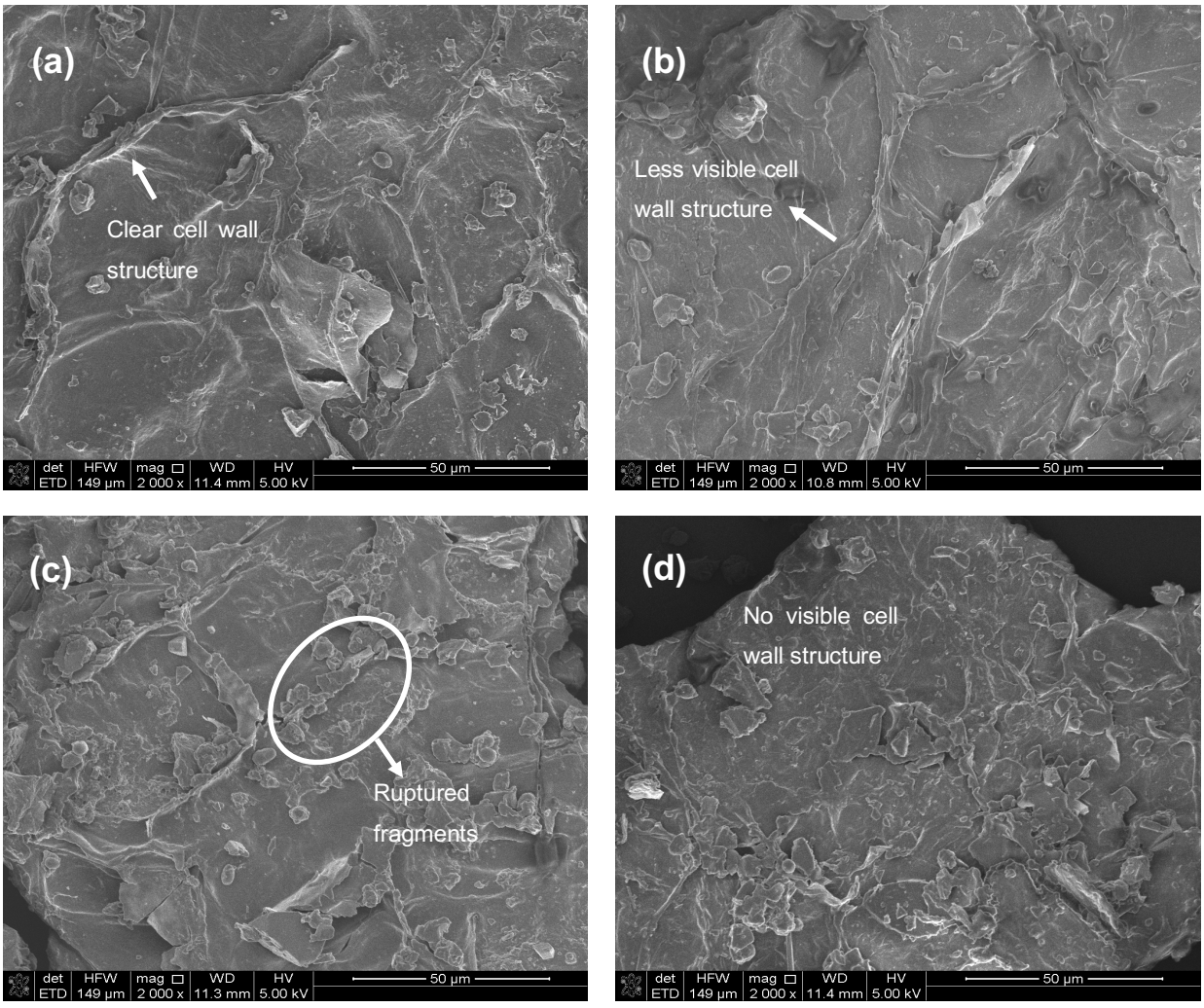

Figure 3.3 SEM images of grape pomace treated by HVACP at 60 kV for (a) 0 min (control), (b) 5 min, (c) 10 min, (d) 15min.

3.3.3 Surface wettability of grape peels and drying kinetics of grape fruit

Water contact angle measurement was taken to determine the surface hydrophilicity of grape peels under different plasma exposure times, as shown in Figure 3.4. The control grape peel had a water contact angle of 86º, representing a more hydrophobic surface. After exposing to HVACP, the contact angle significantly decreased with the treatment time by 29.1% (5 min) −41.9% (15 min), indicating the formation of more hydrophilic surfaces. Huang et al. (2019) exposed fresh white grapes to atmospheric plasma jet at 500 W and also found 12.5–41.4%

reduction in the water contact angle of grape skin. In the study of Grzegorzewski et al. (2010) on the effect low-pressure oxygen plasma on the surface wettability of lettuce, a time-dependent reduction in its water contact angle from 88º to 34º after 180 s was also observed.

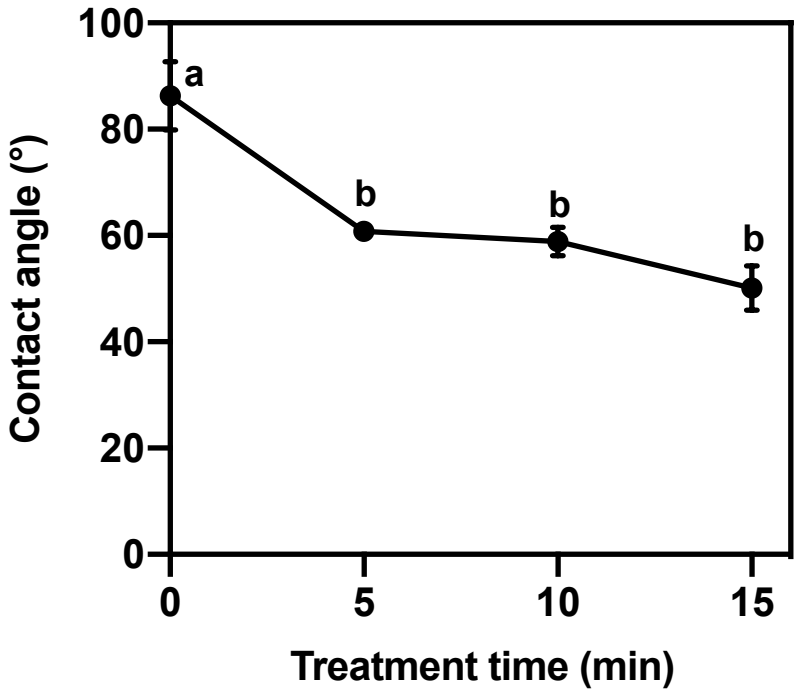

Figure 3.4 Water contact angles of control (0 min) and HVACP-treated grape peels. Different letters represent significant differences among groups ($p < 0.05$).

The outermost layer of grape skin is cuticle, composed of cutin and covered by hydrophobic waxes (Pinelo et al., 2006). The reactive species in plasma could degrade the epidermis waxy layer and thus make plant surfaces more hydrophilic. In addition, the reduced surface wettability observed in the HVACP-treated GP can be attributed to changes in surface chemical composition and increase in surface energy. As mentioned in Section 3.3.1, the air residue in the plasma chamber was also ionized to produce reactive oxygen and nitrogen species in plasma discharge. The reactive oxygen species can convert C=O bond to C-O and C-OH bonds while the reactive

nitrogen species can break C-C and/or C-H bonds as well as activate treated surfaces to form new hydrophilic functional groups, including amine, amide, carboxyl and carbonate (Yang et al., 2005; Fatyeyeva et al., 2014). These reactions could synergistically increase the O and N contents of treated surfaces while decreasing the C content, and thus create more hydrophilic surfaces. Moreover, plasma reactive species have widely proved to have etching effect on exposed surfaces to increases surface energy as well as surface roughness, resulting in reduction in their hydrophobicity (Meng et al., 2016; Van Deynse et al., 2016; Wang et al., 2016).

Grape skin features a hydrophobic layer and acts as barriers to protect the inner plant tissue from dehydration, mechanical injuries, pathogen invasion, UV damage as well as supports the plant integrity (Pinelo et al., 2006). The formation of more hydrophilic grape peels owing to degradation of their epidermis layer caused by reactions with plasma reactive species can increase the water permeability of grape surface and facilitate water diffusion from interior matrix. Figure 5 presents the drying curves of grape fruit at 80 ºC over 23 h. It is shown that HVACP treatment accelerated the grape drying, and the drying rate increased with the treatment time. The most marked increases in the moisture loss were observed at the fifteenth hour, of 11.4%, 21.9% and 23.7% ($p < 0.05$) for the samples treated for 5, 10 and 15 min, respectively. Similar enhancement in drying rate was reported on plasma-treated white grapes (Huang et al., 2019). The result of accelerated water diffusion suggests the potential of HVACP for assisting in migration of water-soluble compounds in GP during extraction.

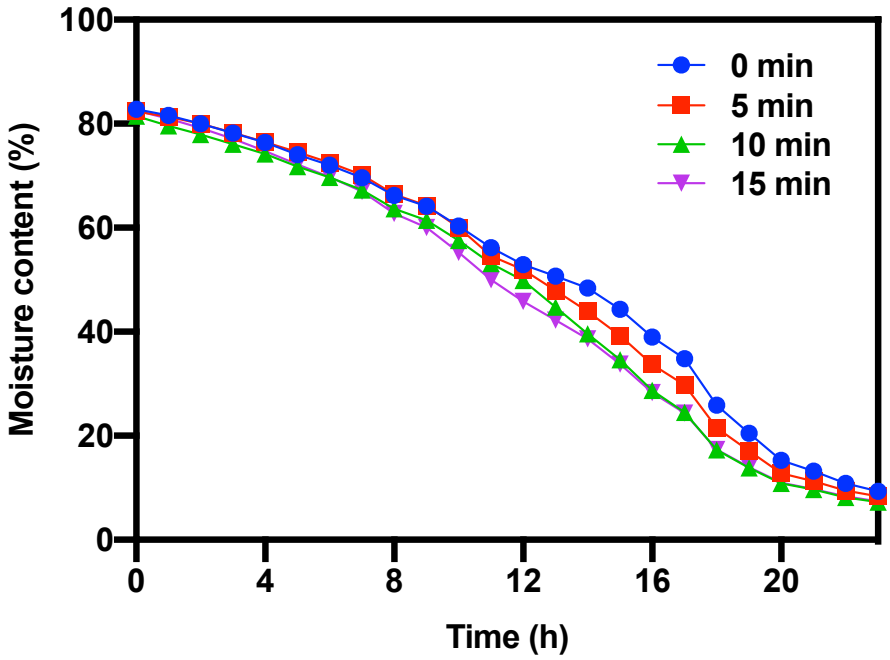

Figure 3.5 Drying curves of control and HVACP-treated black seedless grapes at 80 ºC. Each point represents the mean value of triplicate tests.

3.3.4 Total phenolic and total anthocyanin contents of grape pomace extracts

Figure 3.6 exhibited the effect of HVACP with different treatment times on the TPC and TAC (expressed based on dry mass) of the GP extracts. The phenolic extracts from the control had a TPC of 23.94 mg gallic acid equivalent (GAE)/g. Both the 5-min and 15-min treatment groups showed significantly higher phenolic extraction yields compared to the control, by 19.8% and 22.8%, respectively. The 10-min group exhibited a slightly higher average TPC (26.56 mg GAE/g), but with no statistically significant difference from the control. In the study of Rodríguez et al. (2017), the TPC of cashew apple juice significantly increased after indirect nitrogen-cold plasma treatment, and Huang et al. (2019) also found a higher TPC in plasma jet-treated white grapes. However, different effects of cold plasma on TPC were also reported. The TPC of prebiotic orange juice decreased significantly after DBD plasma treatment at 70 kV

(Almeida et al., 2015), and a similar reduction in TPC was also observed in white grape juice treated by HVACP at 80 kV for 1−4 min (Pankaj et al., 2017).

Figure 3.6 also shows the TAC of the GP extracts after the HVACP treatment. With the same trend as the TPC, the TAC increased by 30.9% after 5 min and by 22.3% after 15 min compared to the control, and no significant improvement was shown at 10 min. The positive effect of cold plasma on anthocyanin was also presented in previous studies that argon-plasma jet of different powers enhanced the TAC of pomegranate juice by 21−35% (Bursać Kovačević et al., 2016), and the concentrations of various anthocyanins in red wine, including delphinidin, petunidin, petunidin-3-glucosides and malvidin-3-glucosides (Sainz-García et al., 2019).

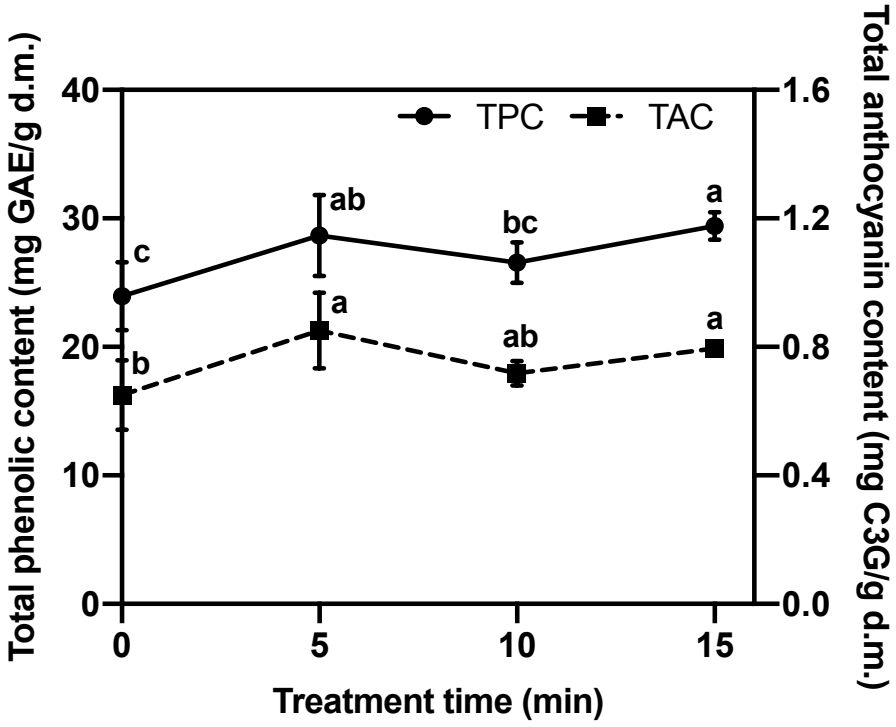

Figure 3.6 Total phenolic content (solid line) and total anthocyanin content (dash line) of extracts from control and HVACP-treated grape pomace at 60 kV. Different letters represent significant differences among groups ($p < 0.05$).

The higher PC extraction yields can be because the surfaces of the HVACP-treated GP have been modified to a more hydrophilic status by the plasma reactive species, as described in the previous section. In addition, the HVACP-induced ruptures in the GP cell structures (Figure 3.3) can reduce the spatial hindrance for molecule movements and favor the release of free PC localized in the cell vacuole and cytoplasm to extracting solvents (Pinelo et al., 2006). The reactive species of plasma also have the potential to break covalent bonds hence release the PC that are covalently bound to cell wall polysaccharides (Ben Belgacem et al., 2017; Çankaya, 2018), which contributed to the higher extraction yields observed.

It is important to point out that although 10 min of HVACP treatment did cause GP cell disruption (Figure 3.3c) as well as increase the surface wettability of grape peels (Figure 3.4), it showed an insignificant effect on both the TPC and TAC of the GP extracts. These findings suggest the negative effect of HVACP on PC extraction, which could be the oxidation caused by plasma reactive species considering their strong oxidizing power (Kim et al., 2019). Therefore, the interacting mechanisms between the HVACP and GP could include three stages. The majority of plasma reactive species only modified GP surfaces over the first 5 min, facilitating the release of free PC during following extraction. Extending the treatment led some reactive species to diffuse through the ruptured cell walls and oxidize part of the free PC in GP cells (Misra et al., 2014; Pankaj et al., 2017), which counterbalanced the positive effect of surface modification on PC extraction. Therefore, the samples with 10-min treatment showed similar extraction yields to the control. More reactive species diffusing into the intracellular matrix of GP during prolonged treatment could provide sufficient energy to cleave and release bound PC, hence the PC extraction yields increased again at 15 min. Further studies are needed to investigate the competitive effects of surface modification and oxidation by HVACP on

extraction of bioactive compounds in order to optimize the treatment time with maximum extraction yields.

3.3.5 Antioxidant activity of grape pomace extracts

Figure 3.7 shows the AA (expressed based on dry mass) of the extracts from GP treated by HVACP with different times. Regardless of the treatment time, the HVACP significantly increased the AA of the phenolic extracts. The treatments for 5 and 15 min exhibited larger increases, by 29.0 and 34.7%, respectively, however, the value slightly decreased between 5 and 10 min. Although the AA increment can be explained by the increasing TPC and TAC of the GP extracts (Figure 6a), it is important to notice that the AA to TPC ratio (i.e., specific AA of phenolics) was 1.68, 1.81, 1.77, and 1.84 for the control, 5-min, 10-min, and 15-min treatment groups, respectively. The increased specific AA indicates that the higher AA of the HVACP-treated samples is not only due to their higher TPC and TAC, but also because the PC with higher AA could be more easily extracted from the GP with HVACP treatment. Kim et al. (2017) reported AA improvement in onion powder treated by microwave-powered cold plasma at 400 W for 40 min. The same type of cold plasma was also found to increase the free radical scavenging activity of mandarin peel against DPPH (Won et al., 2017).

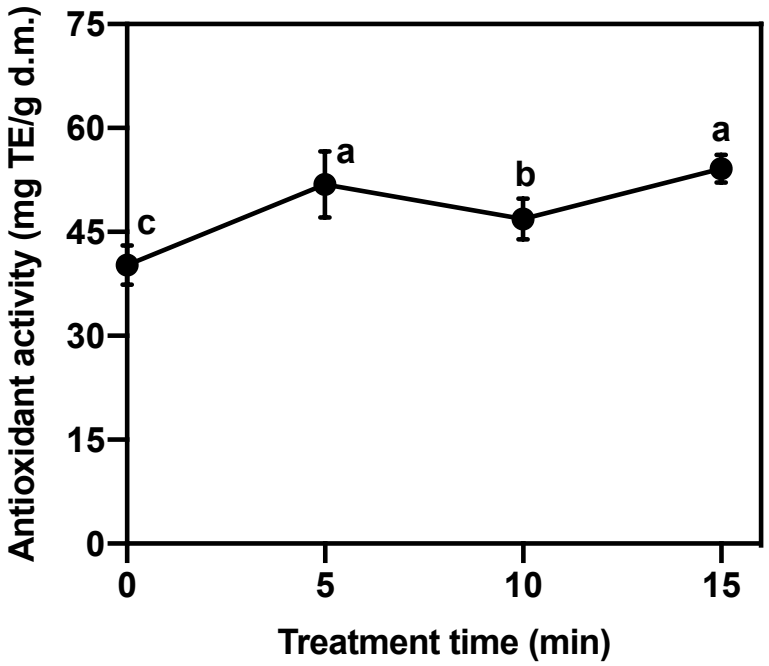

Figure 3.7 Antioxidant activity of extracts from control and HVACP-treated grape pomace at 60 kV. Different letters represent significant differences among groups ($p < 0.05$).

3.3.6 Phenolic compound composition in grape pomace extracts

UPLC chromatograms of hydrolyzed phenolic extracts detected at 290 nm, 350 nm and 520 nm are displayed in Figures 3.8a, 3.8b and 3.8c, respectively. Phenolic acids (gallic acid and protocatechuic acid), non-anthocyanin flavonoids (quercetin) and anthocyanidins were identified by comparing the UV spectra and retention time with their standards. The phenolics profile of the control GP extract is consistent with previous studies (Kammerer et al., 2004; Ramirez-Lopez & DeWitt, 2014). Table 3.1 summarizes the concentration of each identified phenolic compound. Compared to the control, the extracts from the GP treated by HVACP for 5, 10 and 15 min had 15.1%, 10.1% and 25.2% higher quercetin concentrations, respectively. This is consistent with the findings of Pankaj et al. (2017) that the total flavonols content of white grape juice was enhanced by 1−4 min exposure to 80-kV-HVACP due to incorporation of hydroxyl

groups into aromatic ring structure. For the anthocyanidins, 15 min of HVACP treatment showed significant increases in the concentrations of delphinidin, cyanidin, petunidin and malvidin, and 5-min treatment only enhanced the delphinidin content significantly, whereas the 10-min group did not show significant improvements. In contrast, the total anthocyanidin concentration increased with HVACP treatment time from 0.296 mg/g dry mass (control) to 0.347, 0.371 and 0.403 mg/g at 5, 10 and 15 min.

Flavonoids are bound to the cell walls of fruit peel with lower energy, which could be cleaved more easily by the etching process induced by plasma reactive species (Rodríguez et al., 2017; Won et al., 2017). Therefore, flavonoids are more extractable compared to phenolic acids. Additionally, flavonoids, such as quercetin, cyanidin and delphinidin have much higher antioxidant capacity compared to other PC (Rice-Evans et al., 1997), which could explain the higher AA of the extracts from the HVACP-treated GP. However, other unidentified antioxidants, including some other PC and vitamin C, may also be associated with the AA of GP extracts, which need to be further analyzed.

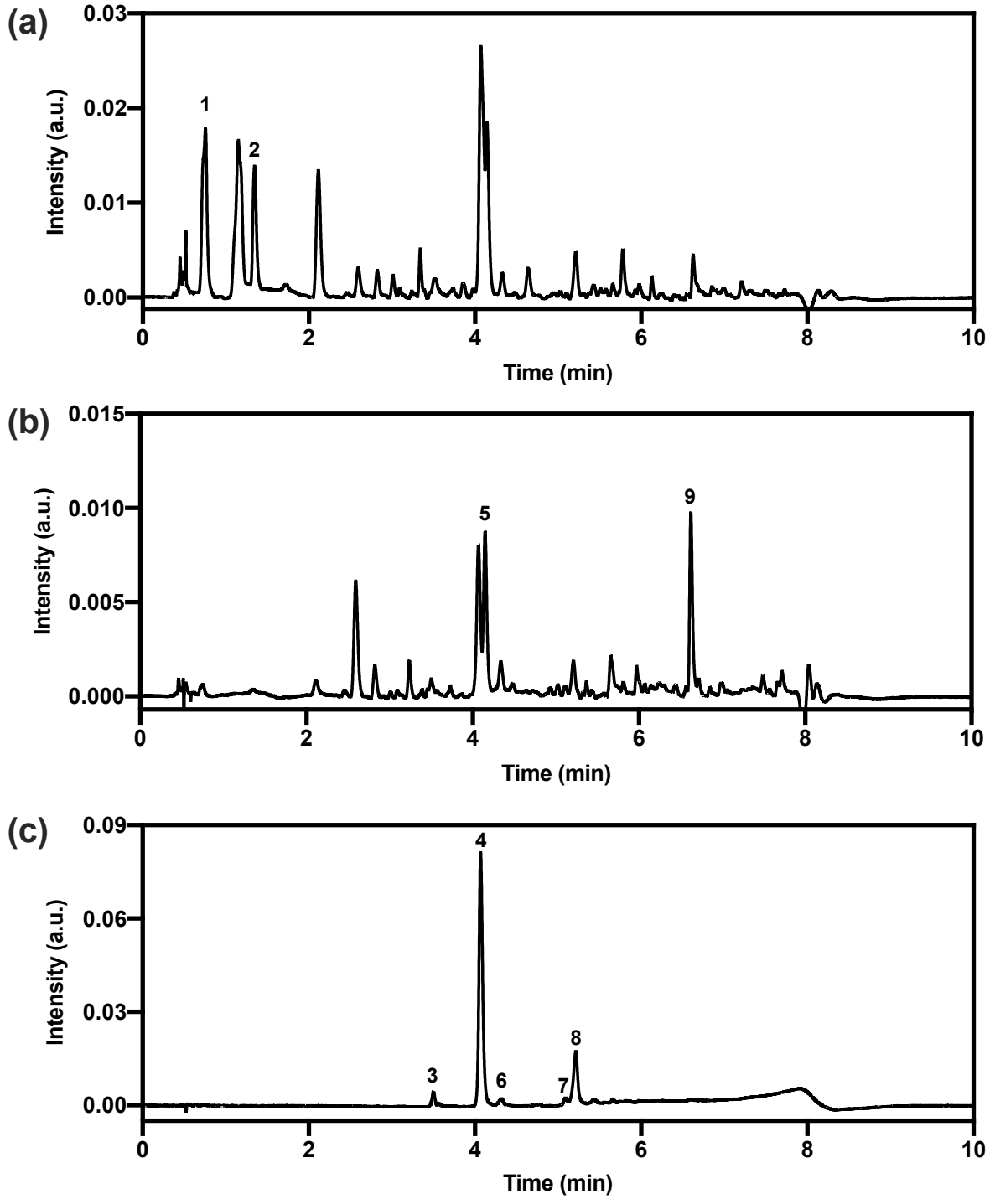

Figure 3.8 UPLC chromatogram of phenolic compounds extracted from grape pomace detected at (a) 290 nm, (b) 350 nm and (c) 520 nm. Peaks represent (1) gallic acid, (2) protocatechuic acid, (3) delphinidin, (4) cyanidin, (5) 7-hydroxycoumarin (internal standard), (6) petunidin, (7) peonidin, (8) malvidin, (9) quercetin.

Table 3.1 Phenolic compound composition of extracts from control and HVACP-treated grape pomace

Treatment time (min)	Phenolic compounds (mg/g d.w.)							
	Gallic acid	Protocatechuic acid	Quercetin	Delphinidin	Cyanidin	Petunidin	Peonidin	Malvidin
0	0.423 ± 0.031^{b}	0.216 ± 0.027^{a}	0.119 ± 0.014^{b}	0.091 ± 0.008^{b}	0.867 ± 0.119^{b}	0.083 ± 0.006^{b}	0.070 ± 0.004^{a}	0.367 ± 0.048^{b}
5	0.455 ± 0.045^{ab}	0.248 ± 0.012^{a}	0.137 ± 0.001^{ab}	0.108 ± 0.003^{a}	1.023 ± 0.090^{ab}	0.090 ± 0.001^{b}	0.077 ± 0.005^{a}	0.438 ± 0.038^{ab}
10	0.419 ± 0.019^{b}	0.252 ± 0.025^{a}	0.131 ± 0.006^{ab}	0.104 ± 0.004^{ab}	1.072 ± 0.042^{ab}	0.096 ± 0.002^{ab}	0.087 ± 0.008^{a}	0.494 ± 0.036^{ab}
15	0.572 ± 0.076^{a}	0.266 ± 0.013^{a}	0.149 ± 0.004^{a}	0.119 ± 0.007^{a}	1.170 ± 0.049^{a}	0.108 ± 0.008^{a}	0.085 ± 0.013^{a}	0.534 ± 0.069^{a}

All analyses were conducted in triplicate. Data values with different letters in the same column were significantly different ($p < 0.05$).

3.4 Conclusion

This is the first study applying cold plasma to modify grape processing byproducts, aiming to enhance bioactive compounds recovery. The results prove that HVACP treatment is a promising method to change GP surface properties and increase PC extraction efficiency. Disruption of cell structure as well as formation of hydrophilic surface of GP were observed after exposing to HVACP, resulting in higher PC extraction yields. The non-monotonous increases in TPC and TAC suggest potential PC oxidation associated with plasma reactive species. HVACP treatment also improved the AA of GP extracts by producing different PC profiles with a higher concentration of anthocyanin.

More treatment times need to be tested in order to provide scientific insights into the mechanisms and kinetics of cold plasma-induced surface modification and bioactives oxidation in plant materials. Furthermore, other operating parameters of cold plasma, such as working gas and voltage, have to be studied to determine the optimal treatment for PC extraction. The findings of this study can serve as the groundwork to scale up the HVACP-assisted extraction process for valorizing more agricultural byproducts with high nutritional values while mitigating associated environmental burdens.

Acknowledgements

This work was supported by the USDA National Institute of Food and Agriculture, Hatch project 014964.

References

Almeida, F. D. L., Cavalcante, R. S., Cullen, P. J., Frias, J. M., Bourke, P., Fernandes, F. A. N., & Rodrigues, S. (2015). Effects of atmospheric cold plasma and ozone on prebiotic orange juice. *Innovative Food Science & Emerging Technologies, 32*, 127–135. https://doi.org/10.1016/j.ifset.2015.09.001

Aspé, E., & Fernández, K. (2011). The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from Pinus radiata Bark. *Industrial Crops and Products, 34*(1), 838–844. https://doi.org/10.1016/j.indcrop.2011.02.002

Ben Belgacem, Z., Carré, G., Charpentier, E., Le-Bras, F., Maho, T., Robert, E., Pouvesle, J.-M., Polidor, F., Gangloff, S. C., Boudifa, M., & Gelle, M.-P. (2017). Innovative non-thermal plasma disinfection process inside sealed bags: Assessment of bactericidal and sporicidal effectiveness in regard to current sterilization norms. *PLoS ONE, 12*(6). https://doi.org/10.1371/journal.pone.0180183

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bursać Kovačević, D., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Režek Jambrak, A., & Herceg, Z. (2016). Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chemistry, 190*, 317–323. https://doi.org/10.1016/j.foodchem.2015.05.099

Çankaya, N. (2018). Recent Research in Polymerization. BoD – Books on Demand.

Faller, A. L. K., & Fialho, E. (2009). The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. *Food Research International, 42*(1), 210–215. https://doi.org/10.1016/j.foodres.2008.10.009

Fatyeyeva, K., Dahi, A., Chappey, C., Langevin, D., Valleton, J.-M., Poncin-Epaillard, F., & Marais, S. (2014). Effect of cold plasma treatment on surface properties and gas permeability of polyimide films. *RSC Advances, 4*(59), 31036–31046. https://doi.org/10.1039/C4RA03741C

Fridman, G., Friedman, G., Gutsol, A., Shekhter, A. B., Vasilets, V. N., & Fridman, A. (2008). Applied Plasma Medicine. *Plasma Processes and Polymers, 5*(6), 503–533. https://doi.org/10.1002/ppap.200700154

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Current Protocols in Food Analytical Chemistry, 00*(1), F1.2.1-F1.2.13. https://doi.org/10.1002/0471142913.faf0102s00

Grzegorzewski, F., Rohn, S., Kroh, L. W., Geyer, M., & Schlüter, O. (2010). Surface morphology and chemical composition of lamb's lettuce (Valerianella locusta) after exposure to a low-pressure oxygen plasma. *Food Chemistry, 122*(4), 1145–1152. https://doi.org/10.1016/j.foodchem.2010.03.104

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P. J., & Bourke, P. (2016). Mechanisms of Inactivation by High-Voltage Atmospheric Cold Plasma Differ for Escherichia coli and Staphylococcus aureus. *Applied and Environmental Microbiology, 82*(2), 450–458. https://doi.org/10.1128/AEM.02660-15

Hoffmann, C., Berganza, C., & Zhang, J. (2013). Cold Atmospheric Plasma: Methods of production and application in dentistry and oncology. *Medical Gas Research, 3*, 21. https://doi.org/10.1186/2045-9912-3-21

Huang, C.C., Wu, J. S.B., Wu, J.S., & Ting, Y. (2019). Effect of novel atmospheric-pressure jet pretreatment on the drying kinetics and quality of white grapes. *Journal of the Science of Food and Agriculture, 99*(11), 5102–5111. https://doi.org/10.1002/jsfa.9754

Huang, X.M., Huang, H.B., & Wang, H.C. (2005). Cell walls of loosening skin in post-veraison grape berries lose structural polysaccharides and calcium while accumulate structural proteins. *Scientia Horticulturae, 104*(3), 249–263. https://doi.org/10.1016/j.scienta.2004.09.002

Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol Screening of Pomace from Red and White Grape Varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry, 52*(14), 4360–4367. https://doi.org/10.1021/jf049613b

Keidar, M., Walk, R., Shashurin, A., Srinivasan, P., Sandler, A., Dasgupta, S., Ravi, R., Guerrero-Preston, R., & Trink, B. (2011). Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. *British Journal of Cancer, 105*(9), 1295–1301. https://doi.org/10.1038/bjc.2011.386

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules (Basel, Switzerland), 18*(2), 2328–2375. https://doi.org/10.3390/molecules18022328

Kim, H.J., Nam, G.S., Jang, J.S., Won, C.H., & Kim, H.W. (2019). Cold Plasma Treatment for Efficient Control over Algal Bloom Products in Surface Water. *Water, 11*(7), 1513. https://doi.org/10.3390/w11071513

Kim, J. E., Oh, Y. J., Won, M. Y., Lee, K.S., & Min, S. C. (2017). Microbial decontamination of onion powder using microwave-powered cold plasma treatments. *Food Microbiology, 62*, 112–123. https://doi.org/10.1016/j.fm.2016.10.006

Kodama, S., Thawatchaipracha, B., & Sekiguchi, H. (2014). Enhancement of Essential Oil Extraction for Steam Distillation by DBD Surface Treatment. *Plasma Processes and Polymers, 11*(2), 126–132. https://doi.org/10.1002/ppap.201300047

Li, M., Koecher, K., Hansen, L., & Ferruzzi, M. G. (2016). Phenolic recovery and bioaccessibility from milled and finished whole grain oat products. *Food & Function, 7*(8), 3370–3381. https://doi.org/10.1039/C6FO00760K

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules, 21*(10). https://doi.org/10.3390/molecules21101374

M. Librán, C., Mayor, L., M. Garcia-Castello, E., & Vidal-Brotons, D. (2013). Polyphenol extraction from grape wastes: Solvent and pH effect. *Agricultural Sciences, 04*(09), 56–62. https://doi.org/10.4236/as.2013.49B010

Meng, J., Wang, Y., Wang, Y., & Ding, Z.Q. (2016). Effect of cold atmospheric plasma treatment on hydrophilic properties of fluorosilicone rubber. *Surface and Interface Analysis, 48*(13), 1429–1435. https://doi.org/10.1002/sia.6053

Misra, N. N., Patil, S., Moiseev, T., Bourke, P., Mosnier, J. P., Keener, K. M., & Cullen, P. J. (2014). In-package atmospheric pressure cold plasma treatment of strawberries. *Journal of Food Engineering, 125*, 131–138. https://doi.org/10.1016/j.jfoodeng.2013.10.023

Nastuta, A. V., Topala, I., Grigoras, C., Pohoata, V., & Popa, G. (2011). Stimulation of wound healing by helium atmospheric pressure plasma treatment. *Journal of Physics D: Applied Physics, 44*(10), 105204. https://doi.org/10.1088/0022-3727/44/10/105204

Negro, C., Tommasi, L., & Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology, 87*(1), 41–44. https://doi.org/10.1016/S0960-8524(02)00202-X

Nishime, T. M. C., Borges, A. C., Koga-Ito, C. Y., Machida, M., Hein, L. R. O., & Kostov, K. G. (2017). Non-thermal atmospheric pressure plasma jet applied to inactivation of different microorganisms. *Surface and Coatings Technology, 312*, 19–24. https://doi.org/10.1016/j.surfcoat.2016.07.076

Pan, Y., Zhang, Y., Cheng, J.H., & Sun, D.W. (2020). Inactivation of Listeria Monocytogenes at various growth temperatures by ultrasound pretreatment and cold plasma. *LWT, 118*, 108635. https://doi.org/10.1016/j.lwt.2019.108635

Pankaj, Shashi K, & Keener, K. M. (2017). Cold plasma: Background, applications and current trends. *Current Opinion in Food Science, 16*, 49–52. https://doi.org/10.1016/j.cofs.2017.07.008

Pankaj, Shashi Kishor, Wan, Z., Colonna, W., & Keener, K. M. (2017). Effect of high voltage atmospheric cold plasma on white grape juice quality. *Journal of the Science of Food and Agriculture, 97*(12), 4016–4021. https://doi.org/10.1002/jsfa.8268

Pinelo, M., Arnous, A., & Meyer, A. S. (2006). Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science & Technology, 17*(11), 579–590. https://doi.org/10.1016/j.tifs.2006.05.003

Ramirez-Lopez, L. M., & DeWitt, C. A. M. (2014). Analysis of phenolic compounds in commercial dried grape pomace by high-performance liquid chromatography electrospray ionization mass spectrometry. *Food Science & Nutrition, 2*(5), 470–477. https://doi.org/10.1002/fsn3.136

Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152–159. https://doi.org/10.1016/S1360-1385(97)01018-2

Rockenbach, I. I., Rodrigues, E., Gonzaga, L. V., Caliari, V., Genovese, M. I., Gonçalves, A. E. de S. S., & Fett, R. (2011). Phenolic compounds content and antioxidant activity in pomace from selected red grapes (*Vitis vinifera L*. and *Vitis labrusca L*.) widely produced in Brazil. *Food Chemistry, 127*(1), 174–179. https://doi.org/10.1016/j.foodchem.2010.12.137

Rodríguez, Ó., Gomes, W. F., Rodrigues, S., & Fernandes, F. A. N. (2017). Effect of indirect cold plasma treatment on cashew apple juice (Anacardium occidentale L.). *LWT, 84*, 457–463. https://doi.org/10.1016/j.lwt.2017.06.010

Rousseaux, C. G., & Schachter, H. (2003). Regulatory issues concerning the safety, efficacy and quality of herbal remedies. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology, 68*(6), 505–510. https://doi.org/10.1002/bdrb.10053

Sainz-García, E., López-Alfaro, I., Múgica-Vidal, R., López, R., Escribano-Viana, R., Portu, J., Alba-Elías, F., & González-Arenzana, L. (2019). Effect of the Atmospheric Pressure Cold Plasma Treatment on Tempranillo Red Wine Quality in Batch and Flow Systems. *Beverages, 5*(3), 50. https://doi.org/10.3390/beverages5030050

Seol, Y., Kim, J., Park, S., & Young Chang, H. (2017). Atmospheric Pressure Pulsed Plasma Induces Cell Death in Photosynthetic Organs via Intracellularly Generated ROS. *Scientific Reports, 7*. https://doi.org/10.1038/s41598-017-00480-6

Shashurin, A., Keidar, M., Bronnikov, S., Jurjus, R. A., & Stepp, M. A. (2008). Living tissue under treatment of cold plasma atmospheric jet. *Applied Physics Letters, 93*(18), 181501. https://doi.org/10.1063/1.3020223

Slinkard, K., & Singleton, V. L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American Journal of Enology and Viticulture, 28*(1), 49–55.

Suwal, S., Coronel-Aguilera, C. P., Auer, J., Applegate, B., Garner, A. L., & Huang, J.Y. (2019). Mechanism characterization of bacterial inactivation of atmospheric air plasma gas and activated water using bioluminescence technology. *Innovative Food Science & Emerging Technologies, 53*, 18–25. https://doi.org/10.1016/j.ifset.2018.01.007

Truong, V.D., Deighton, N., Thompson, R. T., McFeeters, R. F., Dean, L. O., Pecota, K. V., & Yencho, G. C. (2010). Characterization of Anthocyanins and Anthocyanidins in Purple-Fleshed Sweetpotatoes by HPLC-DAD/ESI-MS/MS. *Journal of Agricultural and Food Chemistry, 58*(1), 404–410. https://doi.org/10.1021/jf902799a

Tseng, A., & Zhao, Y. (2012). Effect of Different Drying Methods and Storage Time on the Retention of Bioactive Compounds and Antibacterial Activity of Wine Grape Pomace (Pinot Noir and Merlot). *Journal of Food Science, 77*(9), H192–H201. https://doi.org/10.1111/j.1750-3841.2012.02840.x

Van Deynse, A., Morent, R., & De Geyter, N. (2016). Surface modification of polymers using atmospheric pressure cold plasma technology. In Polymer science: Research advances, pratical applications and educational aspects (pp. 506–516). *Formatex Research Center.* http://hdl.handle.net/1854/LU-8088361

Wang, R., Shen, Y., Zhang, C., Yan, P., & Shao, T. (2016). Comparison between helium and argon plasma jets on improving the hydrophilic property of PMMA surface. *Applied Surface Science, 367,* 401–406. https://doi.org/10.1016/j.apsusc.2016.01.199

Won, M. Y., Lee, S. J., & Min, S. C. (2017). Mandarin preservation by microwave-powered cold plasma treatment. *Innovative Food Science & Emerging Technologies, 39*, 25–32. https://doi.org/10.1016/j.ifset.2016.10.021

Xu, L., Garner, A. L., Tao, B., & Keener, K. M. (2017). Microbial Inactivation and Quality Changes in Orange Juice Treated by High Voltage Atmospheric Cold Plasma. *Food and Bioprocess Technology, 10*(10), 1778–1791. https://doi.org/10.1007/s11947-017-1947-7

Yang, C., Lee, S., Wu, J., & Lin, T. (2005). The properties of copper films deposited on polyimide by nitrogen and oxygen plasma pre-treatment. *Applied Surface Science, 252*(5), 1818–1825. https://doi.org/10.1016/j.apsusc.2005.03.182

Zhang, H., Xu, Z., Shen, J., Li, X., Ding, L., Ma, J., Lan, Y., Xia, W., Cheng, C., Sun, Q., Zhang, Z., & Chu, P. K. (2015). Effects and Mechanism of Atmospheric-Pressure Dielectric Barrier Discharge Cold Plasmaon Lactate Dehydrogenase (LDH) Enzyme. *Scientific Reports, 5*(1), 1–12. https://doi.org/10.1038/srep10031

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

4.1 Conclusions

This study has developed a novel cold-plasma-assisted extraction technology to enhance the performance of conventional solvent extraction. This technique is based on atmospheric DBD plasma generated at high voltage. Operating parameters including working gas and treatment time were tested, and the plasma reactive species generated were characterized. The cold plasma-assisted extraction was applied to tomato and grape pomaces, as waste from fruit processing, for recovery of their bioactive compounds, especially PC. The main findings and achievements of this work include:

(i) HVACP treatment created disruptions on plant epidermis cells which could help release free PC from intracellular matrix as well as cleave PC bound to cell wall. HVACP treatment also enhanced the surface hydrophilicity of fruit peels, which accelerated outward diffusion of water through fruit skin, resulting in higher drying rates. These changes became more significant with longer treatment time, and argon and helium showed the most intense effect among the working gases tested.

(ii) Fruit pomaces treated by helium and nitrogen plasmas showed higher PC extraction yield by up to 23%, but prolonged HVACP treatment appeared to have competitive effects of extractability enhancement and PC oxidation.

(iii) Regardless of the working gas, extracts from HVACP-treated fruit pomaces had increased AA (up to 35%) and different PC profiles, especially the higher anthocyanin content in GP extracts.

In summary, as a nonthermal processing technology with low energy cost, the application of cold plasma for biomass pretreatment provides not only a novel tool to improve the extractability of bioactive compounds but also a green alternative to reduce the environmental impacts associated with conventional extraction. When natural bioactive compounds can be more efficiently recovered from agricultural waste/byproducts, they can be utilized as functional food ingredients and nutraceuticals with high nutritional and economic values. Therefore, the successful development of the cold-plasma-assisted extraction technology can help save the waste resulting from fruit processing and reduce environmental burdens. More importantly, this technology has a significant impact on many multi-million sectors of the U.S. economy, including agricultural, food and nutraceutical industries.

4.2 Recommendations for future work

4.2.1 Process optimization

The results of this study indicate that although cold plasma treatment can increase PC extractability, plasma reactive species could degrade some PC and result in a moderate or no increase in the extraction yield. More treatment time periods need to be tested in order to gain better understanding of how plasma reactive species interact with plant cells and bioactives and their kinetics. Some other plasma operating parameters such as voltage, relative humidity, and plasma types (e.g., plasma jet, pulsing arcs) can be further investigated to optimize the cold plasma-assisted extraction process with maximum PC extraction efficiency.

Moreover, the OES results indicate the presence of reactive oxygen and nitrogen species in all non-air plasmas resulting from air residue in the plasma chamber can interfere with the

treatments. Therefore, the DBD plasma device needs to be modified to create more uniform and pure plasma gases.

4.2.2 Process scale-up

While the bench-top experiments have proved that cold plasma treatment is an effective method for extraction enhancement, the results of this study provide promising groundwork for design and construction of a pilot-scale cold plasma-assisted extraction system. Considering the huge quantities of agricultural waste/byproducts generated in the U.S. every year, an integrated cold plasma-based biorefinery is a necessity for recovering different bioactive compounds.