AN ABSTRACT OF THE THESIS OF

Qian Deng for the degree of Master of Science in Food Science and Technology presented on February 11, 2011.

Title: Chemical Composition of Dietary Fiber and Polyphenols of Wine Grape Pomace Skins and Development of Wine Grape (cv. Merlot) Pomace Extract Based Films.

Abstract approved:

______________________________
Yanyun Zhao

The objectives of this project were to investigate the chemical composition of five varieties of wine grape pomace (WGP) skins obtained in the Pacific Northwest of the United States with the emphasis on dietary fiber (DF) and polyphenolic compounds, and further to evaluate the feasibility of developing WGP extract based edible films in terms of their physicochemical, nutritional and antimicrobial properties. Research studied two varieties of white WGP (WWGP) skins (*vinifera* L. cv. Morio Muscat and cv. Muller Thurgau) and three varieties of red WGP (RWGP) skins (*Vitis vinifera* L. cv. Cabernet Sauvignon, cv. Pinot Noir and cv. Merlot). DF was measured by gravimetric-enzymatic method with sugar profiling by HPLC-ELSD. Soluble polyphenols were extracted by 70% acetone/0.1% HCl/29.9% deionized water and measured spectrophotometrically.

The major composition of insoluble DF (IDF) were Klason lignin (7.9-36.1% DM), neutral sugars (4.9-14.6% DM), and uronic acid (3.6-8.5% DM), which weighed more than 95.1% of total DF in all five WGP varieties. RWGP was significantly higher in DF (51.1-56.3% DM) than those of WWGP (17.3-28.0% DM), but lower in soluble sugar (1.3-1.7% DM in RWGP vs. 55.8-77.5% DM in WWGP) (p<0.05).
RWGP contained larger amount of soluble pectin (water soluble, chelator soluble and hydroxide soluble) (5.1-5.6% DM) compared with that of WWGP (3.2-4.1% DM). Compared with WWGP, RWGP had higher values in total phenolic content (21.4-26.7 mg GAE/g DM in RWGP vs. 11.6-15.8 mg GAE/g DM in WWGP) and DPPH radical scavenging activity (32.2-40.2 mg AAE/g DM in RWGP vs. 20.5-25.6 mg AAE/g DM in WWGP) (p<0.05). The total flavanol and proanthocyanidin contents were ranged from 31.0 to 61.2 mg CE/g DM and 8.0 to 24.1 mg/g DM, respectively for the five WGP varieties. This study not only demonstrated that the skins of WGP can be ideal sources of DF rich in bioactive compounds, but also built up the baseline data for developing innovative utilisations of both red and white wine grape pomace skins.

Because of its highest amounts of water soluble pectin (4.5 mg GUAE/g DM) and total phenolic content (25.0 mg GAE/g DM) among five WGP varieties, RWGP (cv. Merlot) was selected to develop WGP extract based films accompanying the study on their physicochemical, nutritional and antibacterial properties. Pomace extract (PE) was obtained by hot water extraction and had a total soluble solid of 3.6% and pH 3.65. Plant based polysaccharides, low methyl pectin (LMP, 0.75% w/w), sodium alginate (SA, 0.3% w/w), or Ticafilm® (TF, 2% w/w), was added into PE for film formation, respectively. Elongation at break and tensile strength of the films were 23% and 4.04 MPa for TF-PE film, 25% and 1.12 MPa for SA-PE film, and 9.89% and 1.56 MPa for LMP-PE film. Water vapor permeability of LMP-PE and SA-PE films was 63 and 60 g mm m⁻² d⁻¹ kPa, respectively, lower than that of TF-PE film (70 g mm m⁻² d⁻¹ kPa) (p<0.05). LMP-PE film had higher water solubility, indicated by the haze percentage of water after 24 h of film immersion (52.8%) than that of TF-PE (25.7%) and SA-PE (15.9%) films, and also released the highest amount of phenolics (96.6%) than that of TF-PE (93.8%) and SA-PE (80.5%) films. PE films showed antibacterial activity against both *Escherichia coli* and *Listeria innocua*, in which approximate 5 log reductions in *E. coli* and 1.7-3.0 log reductions in *L. innocua* were observed at the end of 24 h incubation test compared with the control. In conclusion, wine grape pomace
extract based edible films with the addition of a small amount of commercial polysaccharides showed attractive color and comparable mechanical and water barrier properties to other edible films. The films also demonstrated their antioxidant and antimicrobial functions.

The results from this study provided guidance on the utilizations of WGP skins based on their chemical compositions, and also demonstrate the possibility of developing innovative packaging materials using WGP skins extracts that may be used as colorful wraps or coatings for food, pharmaceutical or other similar applications.
Chemical Composition of Dietary Fiber and Polyphenols of Wine Grape Pomace Skins and Development of Wine Grape (cv. Merlot) Pomace Extract Based Films

by

Qian Deng

A THESIS

submitted to

Oregon State University

In partial fulfillment of the requirements for the degree of

Master of Science

Presented February 11, 2011
Commencement June 2011
Master of Science thesis of Qian Deng presented on February 11, 2011.

APPROVED:

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Major professor, representing Food Science and Technology

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Head of the Department of Food Science and Technology

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My Signature below authorizes release of my thesis to any reader upon request.

______________________________________________________________
Qian Deng, Author
ACKNOWLEDGEMENTS

This research could not be successfully carried out without the supports from people around me. I feel greatly thankful for being in the Department of Food Science & Technology at Oregon State University. I sincerely acknowledge to those who have helped me in this two and half years.

I would like to dedicate my sincere thanks to my major adviser Dr. Yanyun Zhao for advising me throughout my whole master program with constantly encouragement. Without her patient guidance, it is impossible for me to accomplish in the master program. I learned academic knowledge, laboratorial skills, and interpersonal skills from her, which in future will benefit me for lifelong time.

I would like to recognize Dr. Michael Penner for his professional guidance on dietary fiber and chemical composition analysis, letting me to use his equipments, and being my committee member. Many thanks to Dr. Mark Daeschel for providing me with wine grape pomace, allowing me to use his lab equipments, and also being my committee member. I appreciate Dr. Gita Cherian being my graduate council representative and participating in my master program.

I would like to thank Dr. James Osborne for donating wine grape pomace, Dr. Jingyun Duan for teaching me to run microbial test and giving me lots of help in addition of research, Dr. Seth Cohen for generously donating purified wine grape condensed tannins, and Mr. Jeff Goby for helping me profile neutral sugar by HPLC-ELSD. What’s more, I dedicate many thanks to Mr. Jeff Clawson for his time and effort to help me overcome many instrument issues, Ms. Supaporn Sophonputtanaphoca for giving me lots of advice on determining dietary fiber, and Ms. Jooyeoun Jung for determining resistant proteins for me.

Many thanks also go to Dr. Robert McGorrin, and main office ladies, Linda Hoyser, Linda Dunn, Christina Hull, Debby Iola, and Debby Yacas for providing
assistance in every aspect, and my awesome friends in Corvallis who make my life more than just research and give me physical and mental supports continuously.

Last but not the least, I deeply acknowledge my family in China and relatives in the United States for unconditionally supporting me to come here and pursue my M.S. in Food Science and Technology at Oregon State University. Their love always comforts me whenever I am frustrated, makes me strong whenever I am weak, and encourages me to fulfill my goal.
CONTRIBUTION OF AUTHORS

Dr. Yanyun Zhao assisted with the experimental design, data analysis, and writing of each chapter. Dr. Michael H Penner was involved with writing of Chapter 3.
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CHAPTER 1

Introduction

Wine grape pomace (WGP), the byproduct of winery industry, is composed of wine grape skins, seeds, and stems. WGP is a rich source of malate, ethanol, tartrate, citric acid, grape seed oil and dietary fiber (DF) (Arvanitoyannis, et al., 2006). Additionally, due to the pronouncing amount of polyphenols (Katalinić et al., 2000), WGP are reported to be high in bioactive compounds with anticancer, antioxidant, anti-inflammatory, and antimicrobial properties (Pinelo et al., 2006; Arvanitoyannis, et al., 2006; Spigno and De Faveri, 2007; Cowan, 1999; Özkan, et al., 2004). The Pacific Northwest region of the United States are the leading wine grape production states in the country, in which Washington produced 156,000 tons in 2009 and Oregon 40,200 tons in 2009 (USDA-NASS, 2010a,b). The increasing yield of WGP in US Pacific Northwest and their recognized health benefits drove more studies on their chemical compositions for developing value-added applications.

DF, defined as “edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine” (AACC, 2001), is originated from plant cell walls and is revealed to be of great health benefits (Dreher, 2001). WGP of European varieties (cv. Airén, Cencibel, Mango Negro, Prensal Blanc) was reported to be high in insoluble dietary fiber (IDF), condensed tannins, but low in soluble dietary fiber (SDF) (Valiente et al., 1995; Bravo and Saura-Colixto, 1998; Llobera and Cañellas, 2007; Llobera and Cañellas, 2008; Goni et al., 2009). However, little is known about the WGP of US varieties. Therefore, one of the objectives of this study was to characterize DF in two varieties of white WGP (WWGP) (cv. Muller Thurgau, Morio
Muscat) and three varieties of red WGP (RWGP) (cv. Merlot, Pinot Noir, Cabernet Sauvignon) grown in the Pacific Northwest region. Studies on WGP have emphasized on optimizing the recovery of polyphenols from WGP by investigating extraction conditions (Pinelo et al., 2006a). However, it has not come up a universal protocol to extract polyphenolic compounds from WGP which is able to enhance the extraction efficiency, reduce the solvent usage as well as improve the extract purity. Therefore, it is necessary to optimize extraction methods based on previous publications. Because WGP skins weigh 82% of fresh WGP (Jiang et al., 2011) and contain 39 types of polyphenols (Kammerer et al., 2004), this study aimed at characterizing DF, investigating the method of extracting polyphenolic compounds, and determining polyphenolic compounds in WGP skins originated from Pacific Northwest of the United States.

On the other hand, motivated by the increasing demands on environmentally friendly packaging materials, there are more development of edible films and coatings with competitive mechanical and mass barriers (i.e. gas, moisture, flavor, lipid, aroma) properties to traditional plastic wraps (Han and Gennadios, 2005). Plant materials have been used to form edible films and coatings because they not only consist of required film forming materials (i.e. starch, pectin, soluble sugar, moisture, fatty acid, wax) but also some bioactive compounds, such as polyphenols and organic acids. Park and Zhao (2006) successfully developed cranberry pomace extract based edible films with the aid of pectin and plasticizers. WGP skins contain a various amounts of polysaccharides and sugars, as well as bioactive compounds, which led to the hypothesis that WGP extracts can be used to develop innovative edible films with additional nutritional and antimicrobial functions. Based on our best knowledge, no study has investigated nutritional and antimicrobial properties of WGP extracts based edible films. Therefore, the objective of this study was to develop WGP extracts based films by not only investigating their physiochemical properties, but also the nutritional and antimicrobial properties.
Overall, the objectives of this project consisted of two parts. First, the DF and polyphenols of five varieties of WGP skins in the Pacific Northwest region of the United States were studied in order to provide some baseline data for developing further applications of WGP skins. Secondly, WGP (cv. Merlot) extract based edible films were developed and evaluated in terms of their physicochemical, nutritional, and antimicrobial properties.

**Literature cited**


profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). Food Chem. 119, 715-723.


CHAPTER 2

Literature Review

2.1 Overview of dietary fiber

2.1.1 Definition of dietary fiber

The term “dietary fiber” (DF) was firstly introduced by Hipsley (1953), defined as cellulose, hemicellulose, and lignin in food and was then adopted as the remnant of plant cell walls which resists to the alimentary enzymatic hydrolysis in human bodies in 1970s (Trowell 1972; Trowell 1978). The definition of DF has gradually merged from two perspectives: chemical characterizations and physiological functions. American Association of Cereal Chemists (AACC, 2001) suggested the definition of DF as “dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine”. The constituents of DF include non-starch polysaccharides and resistant oligosaccharides, analogous carbohydrates, lignin, and associated plant substances (AACC, 2001). DF is also classified into insoluble DF (IDF) and soluble DF (SDF), in which SDF is water soluble after enzymatic treatments, while the IDF is the insoluble DF after enzymatic treatments (AACC, 2001).

Antioxidant DF (ADF) was first introduced by Saura-Calixto (1998) who defined a product with larger than 50% DF and high antioxidant activities (per gram sample has capacity of inhibiting lipid oxidation ≥ 200 mg vitamin E equivalent by thiocyanate procedure and free radical scavenging capacity ≥ 50 mg vitamin E by DPPH method) (Saura-Calixto, 1998). Wine grape pomace (Saura-Calixto, 1998), guava fruit (Jeménez-Escrig et al., 2001) and the stems and fruits of cactus (Opuntia ficus-indica) (Bensadón et al, 2010) were all identified as the good sources of ADF.
2.1.1.1 Non-digestible carbohydrates

As indicated by AACC (2001), the non-digestible carbohydrates (NDC) include non-starch polysaccharides, resistant oligosaccharides of which the degree of polymerization are within 3~10, and analogous carbohydrates (“i.e., modified cellulose, resistant starches, and synthesized polymers”) (AACC, 2001). The NDC has been characterized from many resources of plant cell walls and some of them are listed in Table 2.1. Cellulose and hemicellulose are the most abundant NDC in nature (Das and Singh, 2004). They belong to IDF fraction and usually come with lignin (Das and Singh, 2004). Additionally, part of NDC is SDF, such as pectin, pectic polysaccharides and inulin. Pectin or pectic polysaccharides are abundant in plant cell walls. For commercial applications, apple and citrus fruits are the major raw materials for the production of pectin (Thibault and Ralet, 2003). Inulin and oligofructans have been promoted as sources of functional foods in the past decade and are mainly extracted from chicory roots for industrial usage (Niness, 1999). Except for the NDC discussed in Table 2.1, some other common plant cell wall materials, such as alginates, carrageenans, and gums are also widely spread in nature.
<table>
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<th>NDC</th>
<th>Degree of polymerization (DP)</th>
<th>Plant sources</th>
<th>Solubility in water</th>
<th>Structure</th>
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<tr>
<td>Cellulose &amp; hemicellulose</td>
<td>DP for cellulose varies, from 30-300 (Isogai et al., 2008); DP for hemicellulose is smaller than cellulose.</td>
<td>Abundant in nature, such as straws (cellulose 30%-57% DM; hemicellulose 15%-29% DM), fruits &amp; vegetables (cellulose 1%-13.4% DM; hemicellulose 4%-36%); cereals (cellulose 2%-12%; hemicellulose ~ 3%) (Das and Singh, 2004).</td>
<td>Cellulose and hemicellulose are not water soluble; but the cellulose derivatives are water soluble.</td>
<td>Cellulose is a linear polymer of which the glucose units are linked by β 1, 4 carbon bonds. Hemicellulose is formed into a shorter and branched chain and includes other sugar residues other than glucose, such as xylose, mannose, galactose, rhamnose and arabinose.</td>
</tr>
<tr>
<td>Pectin</td>
<td>Various</td>
<td>“~35% of primary walls in dicots and non-graminaceous monocots, 2-10% of grass and other commelinoid primary walls; and up to 5% of walls in woody tissue” (Mohnen, 2008)</td>
<td>Yes</td>
<td>Pectins are mainly composed of 1,4 linked-α-D-galacturonic acids as backbone units. Homogalacturonan is a kind of linear structure while rhamnogalacturonan I and rhamnogalacturonan II are hairy structures with the galacturonic acid backbone and other sugar subunit branches (Mohnen, 2008).</td>
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<tr>
<td>Polyfructans (Inulin &amp; Oligofructans)</td>
<td>DP for inulin is 2-6; DP for oligofructans is 2-8 (Roberfroid, 2007).</td>
<td>Presenting in &gt;36,000 plant species (Carpita et al., 1989); chicory roots as the major raw materials for extracting inulin and oligofructans (Niness, 1999)</td>
<td>Highly soluble</td>
<td>They are heterogeneous structures. The fructose units are linked by β 1, 2 carbon bonds in fructose oligomers, polymers, sucrose and glucose residues (Roberfroid, 2007).</td>
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</table>
2.1.1.2 Lignin

“Lignin is a hydrophobic polymer formed through enzyme-mediated radical coupling of monolignols, mainly coniferyl and sinapyl alcohols” (Hatfield and Fukushima, 2005). Lignin is indigestible in intestinal tract and comes with cellulose and hemicellulose in plant cell walls (Hatfield and Fukushima, 2005; Das and Singh, 2004). Generally, lignin takes about 7-13% dry matter (DM) in agricultural residues (straw, hulls, and bagasse); the amount of lignin in fruits and vegetables ranges widely from traceable amount to 35% DM depending on the varieties, such as lemon has 35% DM lignin but potato has only a traceable amount (Das and Singh, 2004).

Although numerous methods have been applied to quantify the lignin in forages, including acid detergent, Klason, and permanganate methods (Hatfield and Fukushima, 2005), the determination of lignin for dietary fiber in foods and plant residue most frequently employs Klason method which is to weigh the residue after acid hydrolysis of insoluble dietary fiber fraction.

2.1.1.3 Associated plant substances

In the plant tissues, there are associated plant substances attached in the lignin and the non-starch polysaccharides such as waxes, phytate, cutin, saponins, suberin, and tannins (AACC, 2001). Those substances have been widely studied and proven to be of various health benefits to human bodies. For example, tannins, presenting widely in nature (i.e. legums, wine, and berries) are usually divided into two groups: water soluble tannins (simple phenolic compounds with low degree of polymerization (DP)) and condensed tannins (a chain of flavonoid units with DP of 2-50). Tannins show antimicrobial, antioxidant, and anti-radical scavenging activities, and are also anticarcinogens (Dokkum et al., 2008). In general, although the amount is smaller than NDC and lignin in plant cell walls, the plant associated substances usually play several bioactive functions on human bodies, thus have attracted increasing attentions from researchers.
2.1.2 Health benefits of dietary fiber

Health benefits of DF have been widely studied and many of them have been proven in recent decades. One of the most important health benefits of DF is to promote the laxation and increase fecal bulks therefore to prevent colon diseases. Physicochemical properties of DF (soluble or insoluble), gut microflora, and human gastrointestinal (GI) tract influence the effects of DF towards human health (Stewart et al., 2010). IDF in cereals, for instance, was revealed to be the effective laxatives (Topping, 2007), while some SDF, such as pullulan, resistant starch, soluble fiber dextrin, and soluble corn fiber are also ideal candidates for promoting colon health (Stewart et al., 2010). On the other hand, high fiber diets showed great influences on preventing coronary heart disease (Anderson et al., 1994; AACC, 2001; Champ et al., 2003). The soluble and viscous DF distributing in foods (apples, barleys, beans, fruits, oatmeals, etc.), for instance, beet fiber, guar gum, pectin, and soy proteins, can effectively reduce the risk of cardiovascular disease by “changing of diet composition, reducing cholesterol adsorption, changing of bile acid synthesis and reducing cholesterol biosynthesis” (Marlett, 2001). Many other health benefits contributed by DF have also been continuously studied, such as weigh regulation to prevent obesity (Chandalia et al., 2000; Howarth et al., 2001), breast and prostate cancer (Marlett, 2001).

Food and Nutrition Board of the National Academy of Sciences (2002) has suggested the dietary fiber intake amounts for different genders during various age groups. Overall, adult males are suggested to digest more DF than adult females, 30-38 g/d and 21-26 g/d, respectively. During the times of pregnancy and lactation, women require a bit higher amount of DF, 28 g/d and 29 g/d, respectively. The suggested DF for children is lower than adults (19-25 g/d). Because DF from natural sources also has various plant associated compounds which are able to provide more health benefits, it is also recommended to intake dietary fiber from natural plant tissues instead of from nutraceutical supplements.
2.1.3 Analysis of dietary fiber

The methods used for analyzing DF vary based on the purposes of the studies. Generally speaking, food stuffs are tested in powder form and DF from food powders are separated into two fractions: SDF and IDF. They are then quantified and/or characterized photometrically, chromatographically, and/or gravimetrically. It is important to quantify the portion of SDF and IDF in food stuffs, not only for the purpose of labeling (Champ et al., 2003), but also the solubility of DF associated with the physiological effects on human health as was mentioned in 2.1.2. American Official Association of Chemists (AOAC) has approved about 7 official methods of DF analysis (Table 2.2), most parts of which focus on the enzymatic treatment and gravimetrical measurement of DF. The enzymatic methods imitate the food digestion in upper alimentary tracts (Champ et al., 2003) so that the measured DF is able to better represent the physiological effects on animal bodies. Therefore, enzymatic methods are usually applied for analyzing DF in foods.
### Table 2.2 Official methods for analyzing dietary fiber from food stuffs

<table>
<thead>
<tr>
<th>Designation*</th>
<th>Title*</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAC 985.29</td>
<td>Total dietary fiber in foods, enzymatic-gravimetric method</td>
<td>Dried food sample is subsequently treated with $\alpha$-amylase, protease and amyloglucosidase. After precipitating by ethanol, the sample is filtrated through a fritted crucible and then weighed to quantify. This method is only applied in the case that only the amount of total dietary fiber (TDF) is taken into consideration.</td>
</tr>
<tr>
<td>AOAC 991.42</td>
<td>Insoluble dietary fiber in foods and food products, enzymatic-gravimetric method, phosphate buffer</td>
<td>Dried food sample is treated with $\alpha$-amylase, protease and amyloglucosidase. The mixture is then filtered and washed by water in order to remove soluble fraction. The insoluble fiber is washed with 95% ethanol and acetone. The insoluble fiber is the weight after subtracting protein and ash.</td>
</tr>
<tr>
<td>AOAC 991.43</td>
<td>Total, soluble and insoluble dietary fiber in foods and food products, enzymatic gravimetric method, MES-Tris buffer</td>
<td>Mes-Tris buffer is specifically used in this methods compared with another methods where phosphate buffer are applied in. Still, the dried food sample is treated with $\alpha$-amylase, protease and amyloglucosidase. Soluble dietary fiber (SDF) is ethanol precipitated, dried and weighed while the insoluble dietary fiber (IDF) is also dried and weighed. TDF is the sum of SDF and IDF.</td>
</tr>
<tr>
<td>AOAC 992.16</td>
<td>Total dietary fiber, enzymatic-gravimetric method</td>
<td>Grounded, dried foods are autoclaved with heat-stable amylase, amyloglucosidase, and protease. The undigested fiber is precipitated by ethanol, filtrated and weighed gravimetrically. Neutral detergent fiber (NDF), on the other hand, is refluxed in the neutral detergent solution, treated with $\alpha$-amylase dried and deashed. The sum of these portions of residue is TDF.</td>
</tr>
<tr>
<td>AOAC 993.19</td>
<td>Soluble dietary fiber in foods and food products, enzymatic-gravimetric method (phosphate buffer)</td>
<td>This method is for determination of soluble dietary fiber (SDF) in vegetables, fruit, and cereal grains. Sample is free from starch by amylohlucosidase and free from protein by protease. SDF is weighed gravimetrically after filtration, ethanol precipitation and drying.</td>
</tr>
<tr>
<td>AOAC 993.21</td>
<td>Total dietary fiber in foods and food products with ≤2% starch, nonenzymatic-gravimetric method</td>
<td>Foods with ≥10% total dietary fiber and ≤2% starch. Dry samples are suspended in distilled water for 90 min at 37 °C to dissolve the soluble compounds. SDF is washed by ethanol while the IDF is weighed gravimetrically.</td>
</tr>
<tr>
<td>AOAC 994.13</td>
<td>Total dietary fiber (determined as neutral sugar residues, uronic acid residues, and Klason lignin), gas chromatographic-colorimetric-gravimetric method (Uppsala method)</td>
<td>The samples are treated with $\alpha$-amylase and amyloglucosidase but without protease treatment then subjected to 80% ethanol to gain SDF. The IDF and SDF are hydrolyzed with 72% sulfuric acid. Neutral sugars are profiled by gas chromatography (GC), pectic polysaccharides are quantified photometrically, Klason lignin is weighed gravimetrically.</td>
</tr>
</tbody>
</table>

* Source: adapted from AACC (2001).
* Source: description of the official method was summarized corresponding to the same code of the official method.
Some studies revealed that the washing step of SDF by 80% ethanol could contribute some error to the accuracy of SDF due to the incompletion of precipitating SDF or introduction of non-fiber compounds, such as organic acids (Mañas and Saura-Calixto, 1993). Therefore, some publications reported to use dialysis tube (12,000-14,000 molecule weight cut-off) to separate small molecules from the SDF solution and then process SDF by freeze drying (Mañas and Saura-Calixto, 1993). In addition, options to profile neutral sugars of DF are not only limited to use gas chromatography (GC) but also high performance liquid chromatography (HPLC) which requires pre-neutralization of H$_2$SO$_4$ hydrolysate (Villanueva-Suárez et al., 2003).

Some other studies not only have focused on the chemical characterization of DF, but also on the extraction of SDF from food plant byproducts (i.e. orange byproduct, peach pomace) and have studied on its rheological properties (Carme Garau et al., 2007; Pagán and Ibarz, 1999).

2.2 Overview of wine grape pomace

2.2.1 Wine grape production and varieties in the Pacific Northwest of the United States

Grapes are classified into wine grapes, table grapes, raisin grapes, sweet juice grapes, and canning grapes in commerciality. The growth of grapes widely spread all over the world and the variety grown is sensitive to the growing climate (primarily heat summation), and directly impacts the brix/acid ratio, total acidity, tannins, and other chemical constituents of the grapes (Winkler et al., 1974a). In US Northwest region, Washington and Oregon grow appreciable amount of wine grapes and also produce promising amounts of wine although the wine industry in California still dominates domestically.
Table 2.3 Summary of 2008 and 2009 wine grape production and average prices in Oregon and Washington

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Wine Grape Production</th>
<th>Average Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Varieties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabernet Franc</td>
<td>200</td>
<td>265</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>1,177</td>
<td>1,232</td>
</tr>
<tr>
<td>Merlot</td>
<td>967</td>
<td>1,125</td>
</tr>
<tr>
<td>Pinot Noir</td>
<td>17,571</td>
<td>21,364</td>
</tr>
<tr>
<td>Syrah</td>
<td>1,120</td>
<td>1,170</td>
</tr>
<tr>
<td>Semillon</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>White Varieties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chardonnay</td>
<td>1,630</td>
<td>2,244</td>
</tr>
<tr>
<td>Gewurztraminer</td>
<td>571</td>
<td>489</td>
</tr>
<tr>
<td>Pinot Gris</td>
<td>5,894</td>
<td>6,718</td>
</tr>
<tr>
<td>Sauvignon Blanc</td>
<td>121</td>
<td>115</td>
</tr>
<tr>
<td>White Riesling</td>
<td>2,633</td>
<td>2,289</td>
</tr>
<tr>
<td>Viognier</td>
<td>307</td>
<td>367</td>
</tr>
<tr>
<td>Total wine grape production or annual average price</td>
<td>34,700</td>
<td>40,200</td>
</tr>
</tbody>
</table>

*The table only reported the wine grapes produced more than 1000 tons per year in Oregon or/and Washington.


The wine grape varieties grown in Oregon and Washington are listed in Table 2.3. Overall, total wine production in Washington in 2008 and 2009 was about 4 times of those of Oregon whilst the average price (dollars per ton) of Oregon’s wine grapes was two-fold as that of Washington’s wine grapes and each variety in Oregon was priced higher than in Washington. Among red wine grapes, Pinot Noir, Cabernet Sauvignon, and Syrah are produced more than 1,000 tons in Oregon. Meanwhile, Merlot, Cabernet Sauvignon, Cabernet, Syrah, and Cabernet Franc are the predominant cultivars in Washington, exceed 2,500 tons in 2008 and 2009. Pinot Noir, well-known as one of the outstanding red varieties in the world (Winkler et al., 1974b),
is identified as signature wine grape in Oregon (>21,000 tons in 2009), followed by Cabernet Sauvignon and Syrah (>1,100 tons). Inversely, Cabernet Sauvignon and Merlot that produce the two mostly world-famous red wines (Winkler et al., 1974b), predominantly grow in Washington, followed by Syrah, Cabernet Franc, and Semillon. In this research, Pinot Noir, Cabernet Sauvignon, and Merlot were chosen as three red wine grape varieties. The berries of Pinot Noir are descriptively small to medium, oval, and black colored. They ripen very early. However, Cabernet Sauvignon’s berries are spherically small in black color and the skins are tough. They usually ripen in late season. Further, the berries of Merlot are round shaped in medium size with bluish black skins (Winkler et al., 1974b).

Regarding to the white wine grape varieties, Pinot Gris is the major one in Oregon, followed by White Riesling and Chardonnay. Nevertheless, Riesling and Chardonnay are the major white wine grapes in Washington which are tenfold as those in Oregon. Furthermore, Gewurztraminer, Pinot Gris, Sauvignon Blanc, and Viognier are also flourished in Washington. In this study, however, Muller Thurgau and Morio Muscat were the two objective white wine grape varieties studied. Across the United States, Oregon is the leading producer of Muller Thurgau since it needs cool climate to maintain the sugar level. The berries of Muller Thurgau are Blanc and ripen early (Schmitz, 2000). The berries of Morio Muscat are white in color, round in shape, and ripen in the middle season (Pool, 2000).

2.2.2 Winemaking procedures (Jackson, 2000a,b)

It has been well recognized that the winemaking in the world can trace back to 6,000 years ago while America only have about 400 years winemaking history (Vine et al., 1997). Winemaking has been improved in the recent couple of centuries. Except the grape varieties, growing regions climates, harvesting time, and wine making procedures vary depending on the requirements of consumers. To make table wines, grapes are harvested, stemmed, crushed, macerated, pressed, fermented and the fermented liquid undergoes some post fermentation steps before bottling, such as malolactic fermentation, maturation, natural clarification, and stabilization (Jackson
Overall, the major differences of red winemaking and white winemaking are that pressing for red winemaking is after fermentation and maceration while it is before fermentation for white winemaking, which directly contributes to the different chemical composition of the red wine grape pomaces (RWGP) from the white wine grape pomaces (WWGP).

Harvesting grapes, stemming, and crushing should be conducted as soon as possible to avoid juice browning and microbial contamination. In the step of stemming, leaves and grape stalks are removed from grape clusters while stems, sometimes, may be left in red winemaking procedures in order to get extra red color, astringent taste, and tannins from the stems. Crushing is carried out immediately after stemming to avoid the browning of released juices and microbial contaminations caused by the damage of grape berries in the previous two steps. Crushing is a procedure for helping the release of juices from the grape berries. After crushing, maceration, fermentation, and pressing are done before getting RWGP, while WWGP is obtained after maceration and pressing. Towards completing the alcoholic fermentation, malolactic fermentation is carried out. Several post-fermentation treatments are also performed before bottling, such as maturation and natural clarification, finishing, and stabilization.

2.2.2.1 Red wine process (Jackson, 2000a)

Red must, the combination of juices, seeds and skins (may also include some stems) are left for fermentation and maceration for red wine. Maceration in red winemaking is for extraction of the pigment and tannin from the must. Time of maceration differs upon the styles of red wine, and usually endures about 3 to 5 days. Along with maceration, fermentation of must is carrying out simultaneously. Yeast, usually *Saccharomyces cerevisiae* and *Leuconostoc oenos*, ferments the sugars in the wine must and converts sugar to ethanol. Meanwhile, the production of ethanol enhances the extraction of the red pigments (anthocyanins) and the solubility of aroma compounds from must, the metabolism of yeast enables to produce various aroma compounds for the wine. The temperature for red wine fermentation is often from 24
to 27 °C, but not necessarily universal, which enables to extract more phenolic compounds from the wine must. After fermentation, pressing is conducted mainly by hydraulic force. RWGP is obtained after pressing. Since the red winemaking process goes through the fermentation which complicated yeast metabolisms involved in, the chemical composition of RWGP differs from that of WWGP. For red wine, malolactic fermentation is often necessary due to the high acidity after completion of fermentation. Except reducing the acidity of wine, malolactic fermentation also modifies the sensory characters and influences the stabilities of microorganisms.

2.2.2.2 White wine process (Jackson, 2000a)

Unlike red wine process, maceration of white grapes is kept as short as possible, maximum a couple of hours. The juice oxidation is the major reason for shortening the maceration time in white wine production. WWGP is obtained after juice pressing. Juice is then subjected to fermentation at low temperature (8-15 °C). What is more, due to the relatively higher pH value for white wine production, adding of malic acid to white wine before malolactic fermentation is required in some cases.

2.2.3 Wine grape pomace (WGP)

WGP, the byproduct from winemaking, is mainly composed of seeds and skins while it is possible to contain stems in RWGP depending on winemaking processes. A very few literature resources are available on the specific chemical composition of WGP other than their polyphenolic compounds.

WGP is a rich source of polyphenols in nature. The distribution of polyphenols in skins and seeds are different. Grape skins are rich in anthocyanins (only in RWGP skins), hydroxycinnamic acids, flavonol glycosides, and flavonols while flavonols mainly existed in grape seeds. Overall, 13 anthocyanins (only in RWGP skins), 11 hydroxylbenzoic and hydroxycinnamic acids, and 13 catechins and flavonols were found in WGP (Kammerer et al, 2004). Therefore, WGP is a diverse and rich source of polyphenols, enabling their bioactivities, such antioxidant, antimicrobial, and anti-cancer activities.
Early, some simple measurements on the chemical composition of WGP other than polyphenols were performed by the researcher in the New York State (Kinsella et al., 1974; Rice, 1976) in order to evaluate potential utilizations of WGP. 6 varieties of WWGP and 5 varieties of RWGP were investigated in terms of their percentage of solid residue (%), moisture content (%), seeds (%), sugar (%), tartrate (%), crude fiber (%), nitrogen (%), phosphorus (%), ash (%), and seed oil (%). Overall, the tartrate (%) of white varieties in average was higher than that of the red varieties. On the other hand, crude fiber (%) of RWGP was higher than that of WWGP. Grape seed oil, however, existed in both of RWGP and WWGP with the close percentage. As the study of chemical composition of WGP getting further, carbohydrates or even specific DF from WGP were studied. Walter (1985) fractionated the hydrocolloidal fractions from WGP (cv. Seyval) by using water, NaOH, EDTA, and HCl independently, and obtained some basic data for extracting hydrocolloids from WGP. Meanwhile, researches on dietary fiber and polyphenols of WGP were carried out in Europe (Saura-Calixto et al., 1991; Valiente et al., 1995; Bravo and Saura-Calixto, 1998). With more attention to nutritional properties of dietary fiber from WGP, the studies in Europe employed protease to treat WGP and then expressed the results in terms of Klason lignin (%), SDF (%), IDF (%), resistant protein (%), uronic acid (%), and polyphenols (%) (Saura-Calixto et al., 1991; Valiente et al., 1995; Bravo and Saura-Calixto, 1998).

Although the wine production is continuously climbing, little information on the WGP from North America is available. Chemical composition data of WGP is essential when comes to develop new applications of this highly valuable byproduct. Therefore, this study aimed to investigate the chemical composition (majorly DF and polyphenols) of five varieties of WGP (cv. Merlot, Carbernet Sauvignon, Pinot Noir, Muller Thurgau and Morio Muscat) which are predominant in the Pacific Northwest region of the United States.

2.2.4 Antioxidant activity of WGP
Since “French paradox” was discovered in 1992 (Renaud et al., 1992), numerous studies have investigated the bioactive functions of WGP. Following that, the studies on the health functions of red wine, especially the antioxidant functions of red wine, have been continuously carried out. Those positive findings stimulated the researches on the antioxidant activity of WGP in which high amounts of polyphenols are left after pressing. Many in vitro methods have been applied to evaluate the antioxidant activity of WGP, such as Folin-Ciocalteau assay (FC assay) for total phenolic compounds, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and Trolox equivalent antioxidant capacity (TEAC) assay for free radical scavenging ability, ferric reducing antioxidant power (FRAP) for reducing power, and oxygen radical absorbance capacity (ORAC) for antioxidant capacity (Ruberto et al., 2007; Kataliníc et al., 2010; Monagas, et al., 2006). Kataliníc (2001) reported that the antioxidant activities of white grape skins were less than those in red grape skins in respect of DPPH and FRAP assay. The relatively high antioxidant activity (162-495 mg vitamin E equivalent/g DM) (Llobera and Cañellas, 2007) of WGP compared with other plant wastes enables WGP to be named as ADF. Except for the in vitro experimental methods, in vivo methods for testing the antioxidant activity of WGP in animals and humans were also performed. Feeding rats with ADF from WGP could effectively reduce the apoptosis by modulating “glutathione redox system and endogenous antioxidant enzymes” (Lópex-Oliva et al., 2010). ADF from WGP was also given to adult panelists and the study found that ADF from WGP was able to reduce the blood pressure and significantly decrease the lipid profile, therefore reduced the risk of cardiovascular disease (Jiménez, et al., 2008).

2.2.5 Antimicrobial activity of WGP

Many researches on the antimicrobial activities of plant based products concluded that phenolics, polyphenols, flavonoids, flavonols, and tannins are among the major sources of antimicrobial agents produced in plants (Cowan, 1999). However, there is no identical finding about whether WGP is more effective on preventing the growth of gram-negative or gram-positive microorganisms. For instance, Kataliníc
(2010) reported that the WGP skin extracts inhibited the growths of both gram-negative and gram-positive microorganisms while some other studies found that the gram-positive organisms are more susceptible to WGP extracts (Corrales et al., 2009; Özkran et al., 2004). Furthermore, it was suggested that some organic acids, such as tannin acid, malic acid, and tartaric acid, performed antimicrobial activities in grape juice and wine (Daglia et al., 2007; Kim et al., 2008). The organic acids left in WGP may still contribute to a part of antibacterial function, but requires further investigations.

2.2.6 Extraction of phenolic compounds from WGP

Efforts on improving the yield and quality of phenolic compounds from WGP have been dedicated to solvent types, ratio of liquid-solvent, temperature, and time (Pinelo, et al., 2006a). Usually, methanol, ethanol, acetone, or ethylacetate is mixed with water in various ratios (Spigno et al., 2007; Spigno and De Faveri, 2007). It is also suggested to use acidified solvents (i.e. acetic acid, hydrochloric acid) (Spigno and De Faveri, 2007) to improve the release of phenolic compounds from plant cell walls thus to yield higher amounts of phenolic compounds (Krygier and Sosulski, 1982; Maisuthisakul and Gordon, 2009). Temperature is another important factor influencing the yield of phenolic compounds from WGP. High temperature but not exceeding 50 °C might enable a higher recovery of phenolic compounds from WGP (Cacace and Mazza, 2003; Pinelo, et al., 2006b). The liquid-solid ratio is also studied to optimize the extraction efficiency while reducing the consumption of organic solvents. The reported extraction time varied between 5 min to 60 h and was related to other extraction conditions (Spigno et al., 2007; Spigno and De Faveri, 2007). Although many publications on extracting phenolic compounds from WGP have been published, no agreement on the most appropriate protocol has been reached. Therefore, it is necessary to investigate the most appropriate extraction procedure of WGP skins in this project so that a similar protocol can be applied to extract phenolic compounds in the future.
2.2.7 Utilizations of WGP

Utilizations of WGP have been widely studied (Table 2.4). The products developed from WGP included fertilizers, organic acids, organic solvents, dietary supplements, ethanol, animal feeds, and food additives. WGP may also be used as the substrates for solid-state fermentation of organic acids, organic solvents, pullulan, and enzymes. The possible applications of WGP for food and nutraceutical industries, as well as other possible utilizations are discussed below.
### Table 2.4 Treatment and usage of grape wastes, physicochemical properties and their utilization

<table>
<thead>
<tr>
<th>Pomace</th>
<th>Treatment</th>
<th>Physicochemical characteristics</th>
<th>Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape waste</td>
<td>Composting of grape waste and hen droppings</td>
<td>Organic matter content</td>
<td>Fertilizer for corn seed</td>
</tr>
<tr>
<td>Grape seed and skin extracts</td>
<td>Fractionation of grape seed and skin extracts from grape waste</td>
<td>Phenol content</td>
<td>Dietary supplements for disease prevention</td>
</tr>
<tr>
<td>Grape waste</td>
<td>Gasification of waste products from grape</td>
<td>Concentrations of unused residues</td>
<td>Gas production for heating purpose</td>
</tr>
<tr>
<td>Pressed grape skin</td>
<td>Composting of solid waste and wastewater</td>
<td>Organic matter content</td>
<td>Fertilizer</td>
</tr>
<tr>
<td>Wine pomace and grape seeds</td>
<td>Lyophilisation and extraction of flavanols</td>
<td>Flavanol content</td>
<td>Dietary supplements, production of phytochemical</td>
</tr>
<tr>
<td>Grape marc, stalks and dregs</td>
<td>Lyophilisation and extraction of polyphenols</td>
<td>Polyphenolic content</td>
<td>Source of flavanols</td>
</tr>
<tr>
<td>Grape skins, seeds and stems</td>
<td>Acidolysis of a polymeric proanthocyanidic fraction of grape pomace in the presence of cysteamine</td>
<td>Flavanol content</td>
<td>Source of flavanols</td>
</tr>
<tr>
<td>Grape seed extract (GSE)</td>
<td>Pre-and post-mortem use of grape seed in feeding experiment</td>
<td>Phenol content</td>
<td>Feedstuff for dark poultry meat</td>
</tr>
<tr>
<td>Grape skin pulp</td>
<td>Fermentation by <em>Aureobasidium pullulan</em></td>
<td>Ethanol precipitate</td>
<td>Pullulan production</td>
</tr>
<tr>
<td>Grape seeds</td>
<td>Solid-state cultivation by <em>Pleurotus</em> sp.</td>
<td>Lignocellulosic content</td>
<td>Laccase production</td>
</tr>
<tr>
<td>Grape pomace</td>
<td>Solid-state cultivation by <em>Pleurotus</em> sp.</td>
<td>Pruning content, high phenolic components and total sugars</td>
<td>Feedstuff for animals</td>
</tr>
<tr>
<td>Wastewater</td>
<td>Electrodiagnosis</td>
<td>Tartaric acid content</td>
<td>Additive in medicines and cosmetics, acidulant compound in soft drinks</td>
</tr>
<tr>
<td>Wastewater</td>
<td>Electrodiagnosis at 60 °C</td>
<td>Tartaric acid and malic acid content</td>
<td>Food and pharmaceutical industries</td>
</tr>
</tbody>
</table>

Source: Adapted from Arvanitoyannis et al. (2006).

Regarded to nutraceuticals, grape seeds, grape extracts, and red wine powder (skins of RWGP) are the basic WGP products in the US market, in which was reported to include 22 types of grape seed products, 5 types of grape extract and 7 types of red
wine skin powder (Shrikhande et al., 2000). Phenolic compounds and unsaturated fatty acids (especially from grape seed oils) are the major functional groups in those dietary supplements. Recent research revealed the possibly medical utilization of WGP because the content of polyphenols might contribute to prevent tooth decay (Kish, 2008).

In food industry, WGP has been evaluated as potential natural food colorants based on its high amount of anthocyanins (Sriram et al., 1999). With the increasing demands for functional foods, the possibility for WGP as food ingredients has been elucidated in several studies. Saura-Calixto (1998) evaluated the possibility of using WGP as “antioxidant dietary fiber” which could be directly applied on various food products (i.e. dairy, cereal, and confectionary products) to enhance the DF content and nutritional value in foods. In a recent study, the WGP powder was applied in minced chicken for reducing the lipid oxidation of raw and cooked chicken hamburgers (Sáyago-Ayerdi et al., 2008). In beverage industry, after distillation and fermentation of WGP, it comes with “Grappa”, a popular and historical alcohol-beverage originating from Italy (Bovo et al., 2009). WGP popsicle has been reported to be successfully made in laboratory (Ishimoto, et al., 2009). For bakery applications, the dry WGP skin powder has also been commercially sold as bakery flour in Canada (Vinifera Fod Life Canada™, Jordan, ON, Canada). The product development of WGP is promising, and not many WGP-incorporated food products are available in the consumer market yet. Therefore, more studies on using WGP for food applications require to be further executed.

Another potential application of WGP is as edible or biodegradable packaging materials. WGP based biocomposite boards have been successfully developed in our laboratory (Park et al., 2010; Jiang et al., 2011) and their application in foods and other agricultural applications as packaging containers are under the way. More fruit and vegetable byproducts, such as cranberry pomace, mango puree, apple puree, have been applied to form edible films and coatings because their extracts naturally contain DF and other film forming materials, as well as some bioactive compounds (Park and
Zhao, 2006; Du et al., 2008a,b; Azeredo et al., 2009). It is believed that WGP extracts can be used as the basic film forming materials to obtain edible films and coatings with antimicrobial and antioxidant properties based on their functional constitutes.

2.3 Edible films and coatings

2.3.1 History of edible films and coatings

Edible films and coatings are formed by food grade materials, either made as a thin-layer sheet as films or as a part of food products when coated directly on the surface of food (Debeaufort et al., 1998). According to the historical record, wax coatings were applied on citrus fruits to prevent the fruit from dehydration in China in 12th and 13th centuries; the soy protein layers from boiled soy milk, called “Yuba”, were applied to preserve foods in Asia in 15th century; edible films were used to coat meat products to avoid shrinkages in 16th century; sucrose was employed to coat the surfaces of tree nut products in 19th century (Debeaufort et al., 1998). In more recent history, edible films and coatings have been applied on a wide range of food products to improve their quality (i.e. appearance, flavor, aroma, nutritional value) and extend shelf-life since 1930s (Debeaufort et al., 1998). Nowadays, with the increasing consumer demands for high quality food products, studies on innovating edible films and coatings with diverse functionalities and excellent mechanical properties continue.

2.3.2 Functions of edible films and coatings for food applications

Edible films and coatings play significant roles on food. Their functionalities may be summarized below.

(a) Edible films and coatings may have mechanical properties that can prevent the physical damage to a certain extent (Han and Gennadios, 2005). However, the mechanical properties of edible films and coatings are usually weaker than those of plastic films (i.e. LDPE, HDPE, EVOH, PVC, and PET) (Han and Gennadios, 2005). Therefore, the study on improving mechanical properties needs to be enhanced and different film forming materials should be continuously investigated to gain better film mechanical properties.
(b) Edible films and coatings are able to regulate mass transfer between foods and the storage environment. Mass transfer can cause adverse effects on food texture, aroma, appearance, nutrient value and food safety (Wu, 2002; Lin and Zhao, 2007). Edible films and coatings can control the exchange of moisture, gas, lipid, aroma and flavor compounds from foods to the environment depending on the nature of film forming materials, and storage conditions (i.e. temperature and relative humidity) (McHugh et al, 1996; Han and Gennadios, 2005). It has been widely recognized that lipid-based edible films and coatings (waxes, lacs and fatty acids) perform better moisture barrier property than that of polysaccharide-based and protein-based edible films (Bourlieu, et al., 2009). Conversely, lipid-based edible films and coatings are less capable to control lipid and gas exchange compared with polysaccharide-based and protein-based films and coatings (Han and Gennadios, 2005; Bourlieu et al., 2009).

Generally, in fresh-cut and minimally processed fruits and vegetables, edible coatings are employed to reduce the moisture loss and control respiration rate of fruits and vegetables (Han and Gennadios, 2005; Vargas, et al. 2008). In cereals and bakery products, edible coatings are applied to control the moisture migration among the different food ingredients in the products (Han and Gennadios, 2005). For the high-fat food products, such as fish (Duan et al., 2010), meat (Caprioli, et al, 2009) and cheese (Cerqueira, et al., 2009), edible films and coatings are widely used to prevent lipid-oxidation and reduce moisture loss (Kerry et al., 2006). In confectionary industry, edible coatings (wax, cellulose gum, whey proteins) are applied for candies and chocolates to prevent “stickiness, agglomeration, moisture absorption, and oil migration” (Debeaufort et al., 1998).

Owing to their capacities of regulating mass transfer, edible films and coatings can also enhance some food processing steps (Han and Gennadios, 2005). For instant, edible coatings applied on fruits and vegetables can reduce the leak-out of soluble compounds from foods and prevent the dehydrating solutions get inside foods during osmotic dehydration (Han and Gennadios, 2005). They can also lower the absorption
of oil during the frying process, as well as decrease the loss of flavor compounds during lyophilization (Han and Gennadios, 2005).

(c) Edible films and coatings can carry active substances to enhance the nutrient value and/or microbial safety of food products. For fruits and vegetables, functional ingredients that are usually added to edible coatings may include antimicrobial agents (essential oils, potassium sorbate, chitosan), antioxidants (citric acid, ascorbic acid, N-acetylcysteine), texture enhancers (calcium chloride, calcium gluconate, calcium lactate), and nutraceuticals (calcium gluconate, vitamin E, ascorbic acid, *Bifidobacterium lactis*) (Rojas-Graü et al., 2009). In meat products, active ingredients including bacteriocin (nisin, lacticin, pediocin), enzymes (glucose oxidase), organic acids, essential oils, and herb extracts may be incorporated into coatings for meat preservation (Coma, 2008).

For food applications, the active compounds should be effectively released from edible films and coatings (Han and Gennadios, 2005). The release speed and amount depend on the interactions between the active compounds and other film forming materials, and the releasing condition (temperature, pH, solution, etc.) (Al-Musa, et al., 1999; Roger, 2006).

### 2.3.3 Film forming materials

In general, the polysaccharides (i.e. pectin, carrageenan, starch) (Maftoonazad et al., 2006; Park and Zhao, 2006; Seol et al., 2009; Kim et al., 2002), proteins (i.e. corn zein, wheat gluten, soy protein, collagen, gelatin) (Cuq et al., 1998), and lipids (i.e. fatty acids, waxes, resins, acetylated glycerides) (Bourlieu et al., 2009) are three basic matrix for forming edible films and coatings (Han and Gennadios, 2005). It has been widely recognized that protein and polysaccharide based films are of relatively high water vapor permeability and stronger mechanical properties while lipid-based films are inverse in both properties (Ruan et al., 1998). To overcome the shortage of each basic film forming material, edible films made from blended materials have been developed lately, such as fatty acid-hydroxypropyl methylcellulose edible films (Hagenmaier and Shaw, 1990), essential oil-alginate-apple puree edible films (Rojas-
Plasticizers, defined as “substantially nonvolatile, high boiling, non-separating substance, which change the physical and/or mechanical properties of other materials” (Banker, 1966) are usually added as film forming materials to reduce the brittleness (Park and Zhao, 2006) and control the mouth feelings of the films (Guilbert, 1986; Kim et al., 2002). However, plasticizers can enhance the diffusion of small molecules since the addition of plasticizers decrease the glass transition temperature of films (Bourlieu et al., 2009). Water, sucrose, corn syrup, propylene glycol, sorbitol, mannitol, xylitol, and glycerol are all used as plasticizers in edible films and coatings (Kim et al., 2002; Han and Gennadios, 2005).

In composite edible films and coatings (emulsion composite or bi-layer composite) containing lipids, emulsifiers (lecithin, Tweens, Spans) are commonly added to the film forming solutions to help the dispersion of lipid droplets in film forming solutions (Bravin et al., 2004; Han and Gennadios, 2005; Pérez-Gago and Krochta, 2005).

Overall, lipids, proteins and polysaccharides compose the major structures of edible films and coatings, plasticizers improve the flexibility, and emulsifiers specially assist lipids incorporating with proteins and polysaccharides. Other functional ingredients such as antimicrobials, antioxidants, texture enhancers, and nutraceuticals may also be incorporated into edible films and coatings for enhancing the physiological, antimicrobial and nutritional properties of the films and coatings.

2.3.4 Overview of edible films and coatings originated from plant materials

2.3.4.1 Sources of plants

The three basic film forming materials (polysaccharides, proteins, lipids) are partially from plant materials. Polysaccharides are abundant in plant materials. Polysaccharides, such as pea starch (Corrales et al., 2009), pectin (Schultz et al., 1949; Oms-Oliu et al., 2008; Pérez et al., 2009), cellulose derivatives (carbomethylcellulose,
hydroxypropylcellulose, and methyl cellulose) (Hagenmaier and Shaw, 1990; Ayranci and Tunc, 2001; Forssell et al., 2002), and xylan from agricultural byproducts (i.e. hulls, sorghum, and wheat straw) (Kayaserilioğlu et al., 2003), have been studied to form edible films and coatings. Plant proteins are also exploited to be good film forming materials. Soy protein and soy protein isolate (Piazza et al., 2009; Denavi et al., 2009; Atarés et al., 2010), corn zein (Kim et al., 2004), and pea protein isolate (Choi and Han, 2002) based films have shown good mass barriers and strong mechanical properties with or without the incorporation of other film forming materials. Plant is also a rich source of lipids. Rice bran wax has been used to coat candy and coumarone indene resin for fresh citrus fruits (Bourlieu et al., 2009). In addition, most parts of fatty acids are originally from plant materials, such as oleic acid from “olive, peanut, palm, corn, rapeseed and canola” and linoleic acid from “soybean, sunflower, corn and cottonseed” (Rhim and Shellhammer, 2005).

Bioactive compounds produced by plant materials have also been added into edible films and coatings in recent years. Essential oils which “are volatile, natural compounds with a strong odor and formed by aromatic plants as secondary metabolites” (Guimarães et al., 2010), have been added to provide both antioxidant and antimicrobial functions. For instance, cinnamon and ginger essential oils were added to the soy protein isolate based films (Atarés et al., 2010); tea tree essential oil was incorporated into hydroxypropyl methylcellulose to form edible films (Sánchez-González et al., 2009); allspice, cinnamon, and clove bud essential oils were added into apple puree based edible films (Du et al., 2009). Plant extracts added edible films have also been studied, such as ginseng extract added alginate films (Norajit et al., 2010), grapefruit seed extract added algae (Gelidum corneum) films (Lim et al., 2010), gooseberry extract added chitosan films (Mayachiew and Davahastin, 2010), and aqueous oregano and rosemary extracts added gelatin films (Gómez-Estaca et al., 2009a). More plant extracts may be applied with better technologies to extract bioactive compounds from plant tissues. While the addition of bioactive compounds enhance the functionality of edible films and coatings, it may alter their mechanical
and mass barrier properties (Sánchez-González et al., 2009; Du et al., 2009; Mayachiew and Davahastin, 2010; Gómez-Estaca et al., 2009a; Atarés et al., 2010; Norajit et al., 2010; Lim et al., 2010). Therefore, in this project, the WGP extract based edible films were evaluated in respects to their physicochemical, mechanical, nutritional and antimicrobial properties.

The method of utilizing plant tissues film forming materials varies in published studies. Many studies have used commercially purified plant based materials as basic film forming materials, while some other studies directly used the raw plant tissues without purification. For example, mango puree, apple puree, and tomato puree have been directly utilized as film forming materials (Du et al., 2008a,b; Rojas-Graü et al., 2007; Sothornvit and Rodsamran, 2008). In our previous study, cranberry pomace extracts that contain pectin, cellulose, polyphenols, organic acids, minerals and other nutrient compounds were successfully applied to form edible films (Park and Zhao, 2006). WGP has similar chemical composition as that of cranberry pomace, thus was used as basic film forming material in this project.

2.3.4.2 Antimicrobial property of edible films and coatings originated from plant materials

Except for the mechanical, moisture and gas barrier properties, antimicrobial property of edible films and coatings are increasingly studied with the intention to extend the shelf-life of food products without the addition of other chemical preservatives. One of the major studies in developing antimicrobial films and coatings is to incorporate essential oils to enhance the antimicrobial properties. Due to their numerous chemical constituents (terpenes, aromatic compounds and terpenoides), essential oils are cytotoxic to a wide range of microorganism (Bakkali, et al., 2008). Therefore, after properly incorporating with other film forming materials, essential oils incorporated films have shown good antimicrobial activity against the growth of Listeria monocytogenes (Ponce et al., 2008; Du, et al., 2009), Salmonella enterica and Escherichia coli O157:H7 (Du et al., 2009). However, only a few publications studied the antimicrobial activities of phenolic compounds incorporated films. Galangal
extracts added chitosan films effectively decreased the growth of *Staphylococcus aureus* (Mayachiew et al., 2010). Grape seed extracts added soy protein isolate film inhibited the growth of *Salmonella typhimurii*, *Escherichia coli O157:H7*, and *Listeria monocytogenes* (Sivarooiban et al., 2008). With the increasing attention on food safety, it is necessary to investigate the antimicrobial property of edible films and coatings with natural plant bioactive compounds.

### 2.3.4.3 Antioxidant property of edible films and coatings originated from plant materials

Along with the antimicrobial activity, antioxidant activity of edible films has also attracted great research interests, not only due to its ability to elongate the shelf-life but also to provide extra health benefits. Majority of studies on the antioxidant activity of edible films have focused on incorporating essential oils and aqueous soluble plant extracts, such as phenolic compounds and organic acids into film forming materials. Essential oils (oregano and pimento) added milk protein based films were applied to beef muscle for preventing lipid oxidation (Oussalah et al., 2004). Borage extracts increased the antioxidant activities of gelatin based edible films compared to the ones with α-tocopherol and butylated hydroxytoluene (BHT) according to the *in vitro* antioxidant analysis (FRAP, ABTS, and iron (II) chelation activity) (Gómez-Estaca, 2009b). Ginseng extracts (Norajit et al., 2010), grape seed extracts (Lim et al., 2010), and gooseberry extracts (Mayachiew and Davahastin, 2009) were employed as antioxidant bioactive compounds in edible films. However, limited study is available about the WGP skins extracts and their antioxidant properties in edible films or coatings, Therefore, part of this project has targeted to evaluate the antioxidant property of the WGP (skins) extract based edible films.

### 2.3.4.4 Applications of edible films and coatings originated from plant materials in foods

Plant based or plant extract added edible films and coatings have been applied on different food products to extend shelf-life. Fresh-cut or minimum processed fruit and vegetable products tend to be easily spoiled by microorganisms as well as browning discoloration (Rojas-Graü et al., 2009). Plant based polysaccharides,
proteins, and lipids are the basic coating forming materials to extend shelf-life of these products by building up gas barrier (oxygen, carbon dioxide, ethylene, and volatile) and moisture barrier functions (Maria et al., 2008; Lin and Zhao, 2007). Plant originated or plant extract added coatings are also applied on meat products for preventing lipid oxidation and spoilages (Kerry et al., 2006). Plant based or plant extract added edible films are used as food wraps, topping decorators and kids’ snacks (Origami Foods LLC., Stockton, CA, USA). Mango puree (Azeredo et al., 2009), apple puree (McHugh and Senesi, 2000), and tomato puree (Du et al., 2008b) have been made into food wraps for various commercial applications.

The development of WGP based edible films in this study aimed to maximize the utilization of WGP as film forming material to obtain films with promising mechanical and water barrier properties. Therefore, the films could be applied as a packaging material to extend shelf-life and enhance flavor and nutrition of wrapped or coated food products.

2.4 Conclusion

Wine grape pomace is abundant in the Pacific Northwest of the United States as a wine processing byproduct. Studies to investigate its chemical composition are essential before developing their value-added applications. Limited information on the dietary fiber and polyphenols of WGP skins (*vinifera* L. cv. Morio Muscat, cv. Muller Thurgau, cv. Cabernet Sauvignon, cv. Pinot Noir and cv. Merlot) from the Pacific Northwest of the United States is available. Therefore, it is necessary to design the protocol for determining the dietary fiber and associated polyphenols and obtain the chemical composition data of these WGP skins.

Furthermore, WGP extract based edible films are anticipated to have antioxidant and antimicrobial functions according to its high content of polyphenols and organic acids. However, a very few study has developed WGP extract based edible films and investigated their antimicrobial and antioxidant properties simultaneously. The release of the bioactive compounds from film matrix is worthy of
studying as it is directly related to the application of the edible package. Therefore, this project also evaluated the feasibility of using WGP extracts to make edible films by investigating their physicochemical, nutritional and antimicrobial properties.

2.5 Reference


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CHAPTER 3

Chemical Composition of Dietary Diber and Polyphenols of Five Different Varieties of Wine Grape Pomace skins

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Abstract

The skins of two white wine grape pomace (WWGP) and three red wine grape pomace (RWGP) from US Pacific Northwest were analyzed for their dietary fiber (DF) and phenolics composition. DF was measured by gravimetric-enzymatic method with sugar profiling by HPLC-ELSD. Insoluble DF composed of Klason lignin (7.9-36.1% DM), neutral sugars (4.9-14.6% DM), and uronic acid (3.6-8.5% DM) weighed more than 95.5% of total DF in all five WGP varieties. WWGP was significantly lower in DF (18.8-30.3% DM) than those of RWGP (51.1-56.3%), but extremely higher in soluble sugar (55.8-77.5% DM vs. 1.3-1.7% DM) (p<0.05). Soluble polyphenols were extracted by acidified 70% acetone and measured spectrophotometrically. Compared with WWGP, RWGP had higher values in total phenolics content (21.4-26.7 mg GAE/g DM vs. 11.6-15.8 mg GAE/g DM) and DPPH radical scavenging activity (32.2-40.2 mg AAE/g DM vs. 20.5-25.6 mg AAE/g DM) (p<0.05). The total flavanol and proanthocyanidin contents were ranged from 31.0 to 61.2 mg CE/g DM and 8.0 to 24.1 mg/g DM, respectively for the five WGP varieties. This study demonstrated that the skins of WGP can be ideal sources of DF rich in bioactive compounds.

Key words: wine grape pomace (WGP), dietary fiber (DF), polyphenols, radical scavenging activity (RSA)
Introduction

The United States is the 4th largest wine producing region in the world. It is estimated that over 4 million metric tons of grapes are harvested and processed into wine products in the US annually (USDA, 2006) and that amount is expected to increase. Wine grape production in Washington and Oregon was 156,000 and 40,200 tons in 2009, respectively; their production being 2nd and 4th among all states (USDA-NASS, 2010). Therefore, tremendous amounts of wine grape pomace (WGP) are available annually in the Pacific Northwest region of the United States. WGP is primarily composed of seeds, skins and stems, and is commonly used for the extraction of grape seed oils (Mattick and Rice, 1976), the production of citric acid, methanol, ethanol, and xanthan via fermentation (Hang and Woodams, 1985; Hang, et al., 1986; Couto and Sanromán, 2005), and as animal feed (Saunders and Takeda, 1982). The large amounts of WGP available at relatively low cost provide an opportunity for value-added product development through innovative technologies.

Dietary fiber (DF), defined as “edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine” (AACC, 2001), is abundant in plant products such as fruits, vegetables, and grains. DF is widely recognized as being a beneficial component of a healthy diet, its consumption being correlated with reductions in risks associated with cardiovascular disease, cancer, and diabetes (Cho and Dreher, 2001). Furthermore, grape skins, which comprise, on average, 82% of the wet weight of WGP (Jiang et al., 2011), contain multiple types of polyphenols, including 39 types of anthocyanins, hydroxycinnamic acids, catechins, and flavonols (Kammerer et al., 2004). These polyphenols have been claimed to have antioxidant activity (González-Paramás et al., 2004) and inhibit low-density lipoprotein oxidation (Yıldırım et al., 2005).

The DF content of WGP from grape varieties in Spain, such as Manto Negro, Cencibel, and Airén, has been published (Valiente et al., 1995; Bravo and Saura-Calixto, 1998; Llobera and Cañellas, 2007). However, little is known of the
composition of WGP resulting from the processing of grapes common to the US Pacific Northwest. These varieties include Pinot Noir, which accounts for >50% of the total wine grape production in Oregon (USDA-NASS, 2010), and Cabernet Sauvignon and Merlot, the latter two being the top red wine varieties in Washington (USDA-NASS, 2010). The chemical composition of WGP is known to vary depending on the grape cultivar, growth climates, and processing conditions. Therefore, the aim of this study was to characterize the DF and polyphenol fractions of WGP skins common to the US Pacific Northwest. The information obtained from this study will be helpful in the development of value added products derived from such WGP. In this study, skins of WGP were abbreviated as WGP.

Materials and Methods

Sample preparation

Two white wine grape pomace (WWGP) samples, *Vitis vinifera* L. cv. Morio Muscat and cv. Muller Thurgau, were donated by a private winery in Corvallis, Oregon, USA. One red wine grape pomace (RWGP), *Vitis vinifera* L. cv. Cabernet Sauvignon, was from a commercial winery in Kennewick, Washington, USA. The other two RWGP samples, *Vitis vinifera* L. cv. Pinot Noir and cv. Merlot, were received from the Oregon State University Research Winery (Corvallis, OR, USA). All WGP samples were processed in fall 2008, packed in the polyethylene terephthalate pails (Ropak Corp., Cincinnati, OH, USA) and stored at -24 °C until used.

Dietary fiber samples were prepared from the skins contained within the WGP using the procedures of Park et al. (2009). Briefly, WGP was thawed at room temperature, stems and seeds were removed manually and the remaining WGP skins were ground in a disintegrator (M8A-D, Corenco, Inc., Sebastopol, CA, USA) to pass a 0.11 mm mesh screen. The resulting preparation was then dried in an environmental chamber (T10RS, Tenney Environmental, Williamsport, PA, USA) at 10% RH and 70 °C for 48 hours. The dried WGP was then milled (Thomas Scientific, Swedesboro, NJ,
USA) to pass a 20-mesh screen. The resulting powders were stored in Ziploc® storage bags (S.C. Johnson & Son, Inc., Racine, WI, USA) at -24 °C until used.

**Analysis of ash, fat and crude protein contents**

Ash, fat and crude protein contents of the WGP powders were determined at a certified commercial laboratory (Bodycote Testing Group, Portland, OR, USA) and the results were expressed as percentage of dry matter (DM).

**Analysis of soluble sugars**

A representative 0.5 g sample of WGP powder in a 35 mL centrifuge tube was extracted three times in succession with 25 mL of 80% ethanol for 15 min at room temperature in an ultrasonic unit (Branson B–220H, SmithKline Co., Shelton, CT, USA) (AOAC 994.13, 2007). Each extraction was terminated by centrifugation (10,000 × g for 10 min) and collection of the resulting supernatant. The combined supernatants, from the three extractions, were evaporated at 50 °C under vacuum by a rotary evaporator (Brinkmann Instruments, Westbury, NY, USA) to remove ethanol and then diluted to 50 mL with water. The sugar content was then determined by thoroughly mixing 1 ml of the soluble sugar-containing solution with 2 mL 75% H₂SO₄ and 4 mL anthrone reagent (anthrone reagent consisted of 0.5 mg anthrone (Alfa Aesar, Ward Hill, MA, USA) in 250 mL 75% H₂SO₄, incubating the reaction mixture at 100 °C for 15 min, cooling to room temperature, and measuring absorbance at 578 nm (UV160U, Shimadzu, Kyoto, Japan) (Gerhardt, 1994). Absorbance values were converted to “glucose equivalents” using a calibration curve prepared with a D-glucose (Sigma Chemical Co., MO, USA) as specified above. Results were expressed as a percent of WGP DM.

**Fractionation of extractable pectins**

WGP total extractable pectins (TEP) were fractionated into water soluble pectin (WSP), chelator soluble pectin (CSP), and hydroxide soluble pectin (HSP) as described by Silacci and Morrison (1990). WGP powder, 1.0 g, was first homogenized (PT 10-35, Kinematica, Littau, Switzerland) in 20 g deionized water for 10 min. The
homogenate was then filtrated (Whatman No.1 filter paper) and the retentate and filtrate collected. WSP was obtained as the precipitate that resulted from the addition of 95% ethanol to the filtrate (filtrate: 95% ethanol = 1:5) and then allowing it to stand overnight in the refrigerator. CSP was obtained from the water extracted residue by first boiling the residue with 95% ethanol for 10 min and then doing three successive extractions of the resulting residue with 50 mL, 20 mM disodium ethylenedinitrilo tetraacetic acid (Na$_2$-EDTA, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA), pH 8.0. Following each extraction the suspension was filtered and the filtrates combined. The residue obtained from the Na$_2$-EDTA extractions was then extracted with 50 mL of 50 mM NaOH for 15 min at room temperature; the suspension was filtered and the filtrate collected for measurement of HSP.

WSP, CSP, and HSP were quantified as galacturonic acid equivalents (GUAE) (Spectrum Chemical, Co., Gardena, CA, USA) based on a colorimetric assay (AOAC 994.13, 2007) using galacturonic acid for preparation of the calibration curve. Briefly, 250 µL boric acid-sodium chloride solution (content) and 250 µL of sample (or standard) were mixed with 4 mL of 96% H$_2$SO$_4$ and incubated at 70 °C for 40 min. Next, 200 µL of dimethyphenol reagent (100 mg of 3, 5-dimethyphenol in 100 mL of glacial acetic acid; Sigma Chemical Co., MO, USA) was added with mixing and the absorbance read at 400 nm and 450 nm, respectively.

**Determination of dietary fiber**

**Separation of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF).** A representative 0.5 g sample of WGP powder was defatted by two successive extractions with 25 mL petroleum ether for 10 min in an ultrasonic water bath at room temperature; the liquid and solid phases being separated by centrifugation for 10 min at 10,000 × g. The defatted residue was then extracted three times in succession with 80% ethanol, as described above, to remove soluble lower molecular weight saccharides. The resulting residue was dried at 40 °C for 16 h. The dried residue was then treated with 0.0275 mL protease (P-5459, Sigma Chemical Co., MO, USA) in
0.05 M phosphate buffer, pH 7.5, for 30 min at 60 °C (AOAC, 1985; Bravo and Saura-Calixto, 1998). Liquid and solid phases were separated by centrifugation at 10,000 × g for 10 min. The supernatant was saved for determination of SDF. The residue was washed with two portions of 10 mL deionized water; the supernatants from these washings were combined with the supernatant obtained following the protease treatment for determination of SDF. The resulting residue was washed first with 95% ethanol and then twice with acetone followed by drying at 40 °C for 16 h (Prosky et al., 1985). This residue was used for IDF analyses.

**Analysis of SDF.** In order to prevent error caused by precipitating dietary fiber with ethanol (Mañ as, 1994), SDF was separated by exhaustive dialysis of the SDF-containing liquid against deionized water using tubing with a molecule weight cutoff of 12,000-14,000 (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) in 1 L of DI water in the refrigerator. DI water was changed at 4, 16, 28, 30, and 36 h, respectively and the dialysis was finished at 48 h of which the conductivity of dialysate was around 2-6 µS/cm, very close to the value of DI water (~1 µS/cm). The dialyzed SDF preparation was freeze-dried and then acid hydrolyzed in 6% sulfuric acid at 121 °C for 1 h (Bravo and Saura-Calixto, 1998). Uronic acids (UA) in the resulting hydrolysate were quantified by colorimetry as described above. Neutral sugars (NS) were determined by HPLC following neutralization by addition of calcium carbonate. D-(+)-glucose, D-(+)-xylose, D-(+)-galactose, D-(+)-arabinose, and D-(+)-mannose were employed as standards. HPLC was done using a Shimadzu 20A series instrument equipped with Shimadzu-LT II evaporative light scattering detector (ELSD) (Shimadzu, Columbia, MD., USA) and a Biorad Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA, USA). The nitrogen pressure of the ELSD was maintained at 50.6 to 52.0 psi and column temperature at 85 °C. Distilled/deionized water was used as the mobile phase with a flow rate of 0.6 mL/min and injection volume of 20 µL (Sluiter, et al., 2008). The sum of NS and UA was taken as the amount of SDF in WGP. All the results were expressed as percentage per DM.
Analysis of IDF. The dry residue was hydrolyzed in a two-stage manner. The first stage involves the addition of 3 mL 72% sulfuric acid to the residue, with stirring, and subsequent incubation at 30 °C for 1 h. The second stage consists of diluting the first-stage hydrolysate to 2.5% sulfuric acid by the addition of 84 g deionized water, followed by incubation of the resulting suspension at 121 °C for 1 h. The hydrolyzed mixture is then filtrated (fritted crucible; Pyrex® 30 mL M, Corning, Inc., USA). The filtrate is used for quantifying UA and NS as described above. The crucible containing the residue is used for the gravimetric determination of Klason lignin (KL) as described by Sluiter et al. (2008). KL determination was based on mass measurements following drying the residue at 105 °C for 16 h and ashing for 5 h at 525 °C. Resistant protein (RP) in the WGP powder, defined as the protein after protease treatment and acid hydrolysis, was determined by the micro-Kjedahl method using a nitrogen-to-protein conversion factor of 6.25 (AOAC 960.52, 1995). IDF was the total of NS, UA and KL, and expressed as percentage per DM.

Determination of bound condensed tannins (CT)

Residue analogous to that used for the IDF analyses, prepared from 0.5 g WGP as discussed above, was used for determination of bound CT using the method described by Reed (1982). The residue was incubated with 50 mL 5% HCl-butanol (v/v) in a capped Erlenmeyer flask at 100 °C for 3 h. After cooling to room temperature, the absorbance of the liquid phase at 553 nm was determined. Standards for CT are not commercially available; therefore, a CT preparation from red wine grape skin (~80.4% by weight CT) was used as the standard in companion analyses (Kennedy and Jones, 2001). The extinction coefficient used for quantification was based on incubating a 5.0 mg sample of the CT standard with 10 mL 5% acid-butanol at 100 °C for 30 min and subsequently determining the liquid phase absorbance at 553 nm. The bound CT of WGP was expressed as percentage per DM.
Extraction and quantification of soluble phenolic compounds

Phenolics extraction from fresh WGP. Two methods were compared for extraction of soluble phenolics in WGP. Frozen WGP were ground to a fine powder in liquid nitrogen and subsequently used for extraction experiments as WGP. Preliminary data (not shown), as well as a survey of the literature (Spigno and Faveri 2007; Spigno, et al., 2007), indicated that 1:4 (w/v) ratio of WGP to solvent was sufficient for extraction of phenolic compounds, thus both methods were carried out at this ratio. In the first method (method “A”), WGP was extracted with 0.1% HCl/70% acetone/29.9% water (v/v/v) by placing the suspension in an ultrasonic unit for 1 h at room temperature; the temperature of the ultrasonic bath was kept below 45 °C by exchanging water when necessary. The liquid and solid phases were then separated by centrifugation at 10,000 × g for 15 min. This procedure was done three times per sample (total of three extractions per sample). The combined supernatant from the three extractions was concentrated on a rotary evaporator at 40 °C under vacuum and then brought to 50 mL with deionized water and stored at -70 °C until analyzed. In the second method (method “B”), the solvent was 70% acetone/30% water and the extraction was done in an environmental shaker at room temperature with constant agitation (125 rpm). This procedure was done two times per sample (total of two extractions per sample). The combined supernatant from the two extractions was concentrated, brought to volume and stored as in method “A”. The thawed supernatants resulting from extraction methods A and B (referred to below as “extracts”) were used as the starting material for the analysis of total phenolics, antiradical scavenging activity, total flavanol, extractable proanthocyanidins, and anthocyanins.

Analysis of total phenolic content (TPC). The total phenolic content of extracts was measured using the Folin–Ciocalteu (FC) reagent-based colorimetric assay as described by Singleton and Rossi (1965). Phenolic content was calculated as gallic acid equivalents (GAE) and reported as mg/g DM. Briefly, 0.5 mL appropriately diluted extract (or gallic acid standard at 0, 50, 100, 150 or 200 ppm; Sigma Chemical
Co., MO, USA) was mixed with 0.5 mL of 2N FC reagent (Sigma Chemical Co., MO, USA) and 7.5 mL deionized water and allowed to stand for 10 min at room temperature; then 3 mL of 20% (w/v) Na₂CO₃ (Sigma Chemical Co., MO, USA) was added to the reaction mixture and it was placed in a 40 °C water bath for 20 min. After the 20 min reaction period, the samples were cooled to room temperature and the absorbance measured at 765 nm.

**Analysis of antiradical scavenging activity (RSA).** The RSA of extracts was determined by the 1,1–diphenyl–2–picrylhydrazyl (DPPH)-based assay (Kasel Kogyo Co. Ltd, Tokyo, Japan) using ascorbic acid as the calibration standard and reported as mg ascorbic acid equivalents (AAE) per g DM (Brand-Williams, 1995). Briefly, 0.5 mL appropriately diluted extract (or ascorbic acid standard at 0, 0.01, 0.02, 0.03, 0.04 mg/mL, Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) was mixed thoroughly with 1.5 mL of DPPH–methanol reagent (9 mg DPPH in 100 mL methanol) and allowed to stand at room temperature for 5 min prior to measuring the solutions absorbance at 517 nm.

**Determination of total flavanol content (TFC).** The total flavanol content of extracts was determined by the vanillin (Alfa Aesar, Ward Hill, MA, USA) colorimetric assay (Price, 1978) using (+)-catechin hydrate (Sigma Chemical Co., MO, USA) as the calibration standard. A 1.0 mL aliquot of appropriately diluted extract (or the catechin standard at 0, 0.06, 0.12, 0.18, 0.24, 0.30 mg/ml) was mixed with 5.0 mL vanillin reagent (0.5% vanillin in 4% HCl-methanol, w/v). This mixture was allowed to react for 20 min at 30 °C and then the absorbance measured at 500 nm. The blank, included in each assay, was treated as above except the blank-vanillin reagent contained no vanillin. Concentrations were determined based on taking the difference between the absorbance of the corresponding assay and blank samples. TFC was expressed as mg catechin equivalents (CE) per g DM.

**Determination of extractable proanthocyanidins (PAC).** Extractable PAC was measured by the colorimetric assay of Porter (1986) using a red wine grape skin CT powder as the calibration standard. Briefly, 0.5 mL extract (or standard solution)
was mixed (vortexed) with 3 mL acid butanol (50 mL 12 N HCl with 950 mL n-butanol) and 0.1 mL iron reagent (2% ferric ammonium sulfate (EMD Chemicals, USA) in 2 N HCl). The mixture was then placed in boiling water for 50 min followed by the measurement of its absorbance at 553 nm. Extractable PAC was expressed as mg CT equivalents per g DM.

**Analysis of anthocyanin content (ACY).** The ACY content of extracts was determined by the pH differential method (Guisti and Wrolstad, 2001). Briefly, extract was diluted to the same extent in 0.025 M potassium chloride (final pH = 1.0) and in 0.4 M sodium acetate (final pH = 4.5) and the absorbance of the two solutions was measured at 520 nm and 700 nm. A solution of malvidin-3-glucoside (Mvd-3-glu), molar absorptivity of 28,000 L/cm/mol and molar mass of 529 g/mol, was used as the calibration standard. ACY was calculated as:

\[
\text{ACY mg/liter} = \frac{(A_{520} - A_{700})_{\text{pH 1}} - (A_{520} - A_{700})_{\text{pH 4.5}}} {529 \times \text{dilution factor} \times 1000}/28000
\]  
(Thimothe et al., 2007), and expressed as mg Mvd-3-glu equivalents/g DM.

**Data Analysis**

All analyses, with the exception of those specified as being done by a commercial laboratory, were done in triplicate. Results were expressed as mean ± SD. Pair t-test was used to compare the differences between the two phenolic extraction methods (A and B). One way ANOVA was used to analyze the differences among WGP samples based on LSD test at 95% confidence level (SAS 9.1, SAS Institute Inc., Cary, NC, USA).

**Results and Discussion**

**Ash, protein and fat**

Overall, RWGP had higher crude protein, fat and ash contents than those of WWGP (Table 3.1), which was consistent with the results found by Baumgärtel et al.
Among WWGP varieties, Muller Thurgau had higher values in crude protein and fat but lower value in ash than those in Morio Muscat. Regarding to RWGP varieties, Cabernet Sauvignon had relatively high crude protein, fat and ash content, an indication of high amino acids and minerals in the pomace sample.

**Table 3.1 Crude protein, fat and ash content of five varieties of wine grape pomace (WGP)**

<table>
<thead>
<tr>
<th>Composition (% DM)</th>
<th>Muller Thurgau</th>
<th>Morio Muscat</th>
<th>Cabernet Sauvignon</th>
<th>Merlot</th>
<th>Pinot Noir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>6.54</td>
<td>5.38</td>
<td>12.34</td>
<td>11.26</td>
<td>12.13</td>
</tr>
<tr>
<td>Fat</td>
<td>2.64</td>
<td>1.14</td>
<td>6.33</td>
<td>3.35</td>
<td>4.74</td>
</tr>
<tr>
<td>Ash</td>
<td>2.53</td>
<td>3.31</td>
<td>7.59</td>
<td>7.19</td>
<td>6.17</td>
</tr>
</tbody>
</table>

**Soluble sugar**

In this study, soluble sugar was defined as mono- or di-saccharides dissolving in 80% ethanol. The two WWGP varieties had significantly ($p<0.05$) higher soluble sugar contents than those of RWGP, about 56% for Muller Thurgau and 78% for Morio Muscat (Table 3.2), while it was between 1.3-1.7% for RWGP. The high soluble sugar content of WWGP was not reported in previous publications and probably due to different winemaking procedures applied and different varieties of the wine grape. WWGP was obtained right after pressing juice while RWGP was after fermented with juice for several days in order to extract color and polyphenols. Hence, the unfermented juice residue attaching to WWGP rendered the considerably higher amount of soluble sugar compared with those in RWGP. Soluble sugar content of RWGP from this study was relatively lower than those commercial RWGP in Europe, such as Cencibel, Manto Negro, and Blauer Portugieser (Bravo and Saura-Calixto, 1998; Llobera and Cañellas, 2007; Baumgärtel et al., 2007), but closer to the data reported in US wine grape varieties, such as cv. Concord, Ives, Baco Noir, and Cascade (Rice, 1976).
Table 3.2 The major carbohydrate fractions of wine grape pomace (WGP)

<table>
<thead>
<tr>
<th>Composition (%) DM</th>
<th>Muller Thurgau</th>
<th>Morio Muscat</th>
<th>Cabernet Sauvignon</th>
<th>Merlot</th>
<th>Pinot Noir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble sugar</td>
<td>55.77±2.12b</td>
<td>77.53±1.01a</td>
<td>1.71±0.49c</td>
<td>1.34±0.92c</td>
<td>1.38±0.93c</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>28.01±1.36c</td>
<td>17.28±0.21d</td>
<td>53.21±0.38b</td>
<td>51.09±0.58c</td>
<td>56.31±1.47a</td>
</tr>
</tbody>
</table>

Different superscripts in rows indicated the differences among five WGP varieties based on LSD test (p<0.05).

Extractable pectins

Overall, RWGP had higher total extractable pectin (TEP) content (50.6 to 56.4 mg GUAЕ/g DM) than that of WWGP (32.3 to 41.2 mg GUAЕ/g DM) (figure 3.1) (p<0.05). However, the similar trend was not observed in respect to individual fraction of the pectin (Figure 3.1), in which the distributions of three pectin fractions varied depending on the variety of WGP. Among the three fractions of extractable pectins, the highest proportion was CSP (79.47% to 94.50% of TEP). CSP of RWGP was higher than that of WWGP. Cabernet Sauvignon was of the highest value of CSP (about 50 mg GUAЕ/g DM), followed by Merlot and Pinot Noir. For WWGP, CSP of Muller Thurgau was 10 mg GUAЕ/g DM, higher than that of Morio Muscat (p<0.05). Relatively, only small amounts of WSP and HSP were observed in both WWGP and RWGP.

WSP of Merlot was significantly (p<0.05) higher than the values in any other varieties, about 4.5 mg GUAЕ/g DM followed by Pinot Noir with a value of about 2.5 mg GUAЕ/g DM. The smallest proportion was HSP with the lowest amount in Morio Muscat (0.42 mg GUAЕ/g DM) and the highest amount in Merlot (0.89 mg GUAЕ/g DM). Insufficient studies on fractionating pectic polysaccharides of WGP into WSP, CSP and HSP have been reported. According to Silacci and Morrison (1990), winemaking procedures did not have largely impact on the distributions of three fractions except that the WSP might be reduced due to the compression step, and the high CSP indicated that the largest proportion of extractable pectin is cell wall bound pectin.
(a) Water soluble pectin (WSP)

(b) Chelator soluble pectin (CSP)

(c) Hydroxide soluble pectin (HSP)
Total extractable pectins (TEP) from wine grape pomace (WGP) expressed as mg GUAE/g DM. Different letters on the top of each column showed the differences among the varieties ($p < 0.05$).

**Figure 3.1 Fractionation of extractable pectins (water soluble, chelator soluble, hydroxide soluble pectin and total extractable pectins) from wine grape pomace (WGP) expressed as mg GUAE/g DM. Different letters on the top of each column showed the differences among the varieties ($p < 0.05$).**

**Dietary fiber**

TDF (Table 3.2) was the predominant composition in the RGWP but not in the WWGP in which soluble sugar took the largest proportion. TDF of 51.1% to 56.3% on dry matter in the RWGP were similar to those reported by Veliente et al. (1995) and Bravo and Saura-Calixto (1998) (about 50% to 60% of TDF).

Major fraction of TDF was insoluble dietary fiber (IDF) which dominated up to 98.5% of the TDF (Table 3.3). RWGP had significantly ($p<0.05$) higher NS content than those of WWGP. Regarding to NS in IDF, Merlot had at least 9% more than other two RWGP varieties, and Muller Thurgau had 46% higher amount of NS than that of Morio Muscat. Among the NS, glucose was of the highest amount for all five WGP varieties, followed with xylose, while arabinose was traced to be the lowest amount. Since cellulose structured by large amount of glucose subunits, substantial amount of glucose existing in IDF indicated that cellulose was the major constituent of IDF in the WGP. This finding was consistent with the results by Bravo and Saura-Calixto (1998) and Veliente et al. (1995). The ratio of xylose to galactose varied from 0.8 in Morio Muscat to 2.1 in Merlot, which, to some extent, indicated the amount of hemicellulose in IDF. This ratio is within the range of other findings (Valiente et al.,
1995; Bravo and Saura-Calixto, 1998). Based on the fact that the major constituent of hemicellulose in grape skins is xyloglucans, structured by the glucan backbone linked with β (1→4) linkage and 75% of glucose siding with xylose and 35% galactose (Thompson and Fry, 2000), it was predictable that a promising amount of hemicellulose is in the WGP skins.
Table 3.3 Dietary fiber content in five different wine grape pomace (WGP)

<table>
<thead>
<tr>
<th>Dietary fiber</th>
<th>Muller Thurgau</th>
<th>Morio Muscat</th>
<th>Cabernet Sauvignon</th>
<th>Merlot</th>
<th>Pinot Noir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>4.59±0.57c</td>
<td>3.16±0.07d</td>
<td>7.33±0.51d</td>
<td>9.16±0.46d</td>
<td>8.43±0.39a</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.91±0.09c</td>
<td>0.45±0.04d</td>
<td>1.94±0.11b</td>
<td>2.13±0.11a</td>
<td>2.09±0.08a</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.67±0.05c</td>
<td>0.54±0.03d</td>
<td>0.97±0.02b</td>
<td>1.02±0.03b</td>
<td>1.17±0.08a</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.43±0.04c</td>
<td>0.34±0.02d</td>
<td>0.76±0.05c</td>
<td>0.75±0.05e</td>
<td>0.58±0.06b</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.49±0.05c</td>
<td>0.36±0.01d</td>
<td>1.48±0.04e</td>
<td>1.54±0.05a</td>
<td>1.12±0.07b</td>
</tr>
<tr>
<td>NS&lt;sub&gt;IDF&lt;/sub&gt;</td>
<td>7.08±0.80d</td>
<td>4.85±0.04c</td>
<td>12.25±0.39b</td>
<td>14.59±0.39a</td>
<td>13.39±0.37b</td>
</tr>
<tr>
<td>UA&lt;sub&gt;IDF&lt;/sub&gt;</td>
<td>4.81±0.66d</td>
<td>3.64±0.21d</td>
<td>8.53±0.82a</td>
<td>6.62±0.25b</td>
<td>5.14±0.70c</td>
</tr>
<tr>
<td>NS&lt;sub&gt;IDF + UA&lt;/sub&gt;</td>
<td>11.89±1.20c</td>
<td>8.49±0.17d</td>
<td>20.78±0.79a</td>
<td>21.20±0.61a</td>
<td>18.50±0.94b</td>
</tr>
<tr>
<td>KL</td>
<td>15.40±0.27d</td>
<td>7.94±0.06c</td>
<td>31.62±0.48b</td>
<td>28.38±0.67c</td>
<td>36.06±0.75c</td>
</tr>
<tr>
<td>IDF</td>
<td>27.29±1.46d</td>
<td>16.44±0.24e</td>
<td>52.40±0.40b</td>
<td>49.59±0.34c</td>
<td>54.59±1.57a</td>
</tr>
</tbody>
</table>

| Glucose       | 0.20±0.07c     | 0.15±0.04b   | 0.33±0.03b         | 0.38±0.1ab  | 0.43±0.39a |
| Xylose        | 0.02±0.00c     | 0.02±0.01b   | 0.03±0.01ab        | 0.04±0.01b  | 0.02±0.00c |
| Galactose     | 0.12±0.04ab    | 0.13±0.04a   | 0.07±0.03b         | 0.15±0.06a  | 0.18±0.02a |
| Arabinose     | 0.07±0.02c     | 0.11±0.01b   | 0.07±0.02b         | 0.13±0.02ab | 0.16±0.01a |
| Mannose       | 0.05±0.01bc    | 0.01±0.00c   | 0.05±0.06bc        | 0.09±0.03b  | 0.22±0.08a |
| NS<sub>SDF</sub> | 0.46±0.13c     | 0.42±0.10e   | 0.54±0.08e         | 0.78±0.22b  | 1.00±0.10a |
| UA<sub>SDF</sub> | 0.43±0.06b     | 0.26±0.03c   | 0.27±0.04c         | 0.73±0.07a  | 0.72±0.06a |
| SDF           | 0.72±0.14c     | 0.84±0.07c   | 0.81±0.06e         | 1.51±0.14b  | 1.72±0.15a |

| (NS<sub>IDF + NS</sub>)% DM | 7.54±0.74d     | 5.27±0.11b   | 12.79±0.34c        | 15.37±0.48e | 14.39±0.29c |
| (UA<sub>IDF + UA</sub>)% DM | 5.07±0.64d     | 4.07±0.25c   | 8.80±0.79a         | 7.34±0.2b   | 5.72±0.72c |
| TDF % DM      | 28.01±1.36d    | 17.28±0.21d  | 53.21±0.38b        | 51.09±0.58c | 56.31±1.47a |

KL, Klason lignin; NS, neutral sugar; UA, uronic acid; IDF, insoluble dietary fiber; SDF, soluble dietary fiber. IDF = NS<sub>IDF</sub> + UA<sub>IDF</sub> + KL; SDF = NS<sub>SDF</sub> + UA<sub>SDF</sub>; TDF = SDF + IDF. Data were expressed as mean ± SD. The different superscripts in the same row were significant different (p <0.05).
Uronic acid (UA) ranged from 3.6-8.5% (Table 3.3) in the IDF fraction, illustrated that a large proportion of pectic polysaccharides composed of cell wall materials. Similarly, UA of RWGP was 7% to 134% higher than that of WWGP. Cabernet Sauvignon had the highest UA value, while Muller Thurgau had the lowest among the five varieties. Since UA is the backbone of pectic polysaccharides in plant cell walls, it was concluded that RWGP generally had higher contents of pectic polysaccharides. By considering the neutral sugar profiling data, it could be confirmed that WGP skins contain a large amount of homogalacturonan (HG) consisting of backbone galacturonic acids with some xylose and glucose and a small amount of hairy pectic polysaccharides (rhamnogalacturonan I and rhamnogalacturonan II) (Arnous and Meyer, 2009).

Unlikely in IDF, SDF took only small portion of TDF in all WGP varieties. In SDF, the NS was mainly associated with UA to form the pectic polysaccharides. The NS and UA values varied from 0.4% to 1.0% DM and 0.3% to 0.7% DM, respectively. NS in SDF was the lowest in Morio Muscat (~0.4% DM) and the highest in Pinot Noir (1.0% DM), and UA in SDF were the highest in Merlot and Pinot (0.73% DM and 0.72% DM, respectively) while the lowest in Morio Muscat and Cabernet Sauvignon (0.26% DM and 0.27% DM respectively). Glucose was the predominant sugar in all five WGP varieties with the highest in Pinot Noir (0.43% DM) and the lowest in Morio Muscat (0.15% DM), but only 0.02% to 0.04% xylose was detected in the SDF of WGP. The NS data in SDF did not agree with the findings by Bravo & Saura-Calixto (1998) in which only arabinose was detected in SDF of WGP, but agreed with the data from Valiente et al. (1995). That the UA value close to NS might indicate that the hairy pectic polysaccharides were the major portion of the SDF. The low UA content detected in the WGP differed from the work by Bravo and Saura-Calixto (1998) and Valiente et al. (1995), possibly owing to the discrepancies in grape berry maturity at the time of harvest, the WGP varieties, and the winemaking procedures.

Considerable amount of Klason lignin (KL) was observed in IDF fraction of the WWGP, but significantly lower than those in the RWGP (Table 3.3). Pinot Noir
possessed the highest amount of KL among RWGP, 4.4% and 7.7% higher than that of Cabernet Sauvignon and Merlot, respectively. In terms of WWGP, KL of Muller Thurgau was nearly double than that of Morio Muscat. The ratios of KL to TDF ranged from 54.9% in Morio Muscat to 64.0% in Pinot Noir. Differences in lignifications of cell wall materials and wine grape variety contributed to the various KL values in the WGP. The high KL content in IDF of WGP skins make it suitable for various applications, such as phenolic resins and adhesives (Stewart, 2008). In this study, resistant proteins (RP) and bound condensed tannins (CT) were also quantified (Table 3.4).

Table 3.4 Bound condensed tannins (CT) and resistant protein (RP) of insoluble dietary fiber (IDF) residue (% DM)

<table>
<thead>
<tr>
<th></th>
<th>Muller Thurgau</th>
<th>Morio Muscat</th>
<th>Cabernet Sauvignon</th>
<th>Merlot</th>
<th>Pinot Noir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bound CT % DM</td>
<td>8.53±0.33c</td>
<td>6.04±0.09d</td>
<td>16.14±0.24b</td>
<td>16.26±0.58b</td>
<td>19.89±0.32a</td>
</tr>
<tr>
<td>RP % DM</td>
<td>2.30±0.01b</td>
<td>1.50±0.04b</td>
<td>4.52±0.10a</td>
<td>3.65±0.03a</td>
<td>4.92±0.57a</td>
</tr>
<tr>
<td>Bound CT : RP</td>
<td>3.71</td>
<td>4.03</td>
<td>3.57</td>
<td>4.45</td>
<td>4.04</td>
</tr>
<tr>
<td>RP % of crude protein</td>
<td>35.17</td>
<td>27.88</td>
<td>36.63</td>
<td>32.42</td>
<td>40.56</td>
</tr>
</tbody>
</table>

Expectedly, the CT and RP values generally had the same trend as KL. Both bound CT and RP values of the RWGP were higher than those of WWGP. Pinot Noir had the highest amount of bound CT (19.89% DM) and the highest RP (4.92% DM), while Morio Muscat was the lowest in both values, 6.04% DM for bound CT and 1.50% DM for RP.

The ratios of bound CT to RP were quite close among different WGP varieties, from 3.57 to 4.45, indicated that the protein-binding capacity of polyphenols in the WGP skins was not affected by the WGP varieties. The CT-RP matrix formed because the polymeric polyphenols were able to precipitate proteins in the plant cell wall during the grape berry maturation. The CT to RP ratios detected in this study were higher than those described by Bravo and Saura-Calixto (1998), but close to that
published by Llobera and Cañellas (2007). The bound CT in the WGP was significantly higher than those in fruit, fruit peels and dark chocolates (Goñi et al., 2009).

In addition, the percentage RP of crude protein (Table 3.4) was 40.6% for Pinot Noir, 36.6% for Cabernet Sauvignon, 32.4% for Merlot, 35.2% for Muller Thurgau, and 27.9% for Morio Muscat, lower than those described in the previous studies, such as 80.6% in RWGP skins (Cencibel) and 78.6% in WWGP skins (Airén) (Bravo and Saura-Calixto, 1998). In this study, RP was determined after protease treatment and sulfuric acid hydrolysis while the RP in those publications were measured right after protease digestion, which contributed the lower ratios of RP to crude protein than literatural values.

**Effect of different extraction conditions on the soluble phenolic compounds**

Results in the soluble phenolic compounds extracted by using two different extracting methods were reported in Table 3.5. Method A was superior to method B in almost all phenolic compounds for all varieties of WGP ($p<0.05$) except TPC of Morio Muscat and Merlot, ACY of Cabernet Sauvignon and Pinot Noir, and PAC of Morio Muscat. By using method A, TPC values of RWGP and Muller Thurgau increased from 39% to 110%, RSA and TFC values increased from 53% to 125% and 24% to 65%, respectively for all the WGP varieties, and PAC values also remarkably increased from 49% to 130% for all varieties except for Morio Muscut. This study demonstrated the influence of extraction conditions on the yield of phenolic compounds from WGP. First, the ultrasound assisted extraction yielded higher content of polyphenols than the extraction carried out at room temperature with low speed shaking. This observation agreed with results from previous publications (Kalia et al., 2008; Khan, Abert-Vian et al., 2009). Secondly, HCl acidified aqueous solvent extraction enhanced the extraction yield of TPC and RSA but little on ACY ($p$ value was from 0.010 to 0.180), which was consistent with the findings from Maisuthisakul and Gordon (2009) and Vatai et al. (2009). Increased TPC and RSA values as a result of acid hydrolysis solvent extraction might be explained by the fact that the hydrolysis
step helped the release of polyphenols from plant cell wall materials (Krygier and Sosulski, 1982; Maisuthisakul and Gordon, 2009). Finally, the three times extraction might also promote the efficiency of phenolics extraction from the WGP.

**Soluble polyphenols**

Since method A worked better than method B in respect to extraction of phenolic compounds, only results from method A are discussed here. TPC values of RWGP were significantly higher than those of WWGP (p < 0.05) due to the lack of ACY in WWGP. No markedly difference in TPC values between Muller Thurgau and Morio Muscat was observed. Among RWGP, TPC of Pinot Noir was lower than that in Merlot and Cabernet Sauvignon (p<0.05) (Table 3.5), which may be attributed to the thinner fruit skin of Pinot Noir, retaining less TPC.

Table 3.6 shows the total polyphenols reported in previous publications on wine grape pomace, grape berry, and other fruit byproducts. TPCs detected in this study were within the range of the published data (9.7 to 54.0 mg GAE/g DM). The higher TPC values in other publications were owing to inclusion of grape seeds into WGP which possessed higher content of phenolics. The TPC values of fresh wine grape berry were also reported in Table 3.6. TPC of Pinot Noir was 38.4 mg/g based on fresh weigh, which was significantly higher than value from this study (21.4 mg GAE/g DM). The reduction of TPC in WGP was due to the extraction of polyphenols from red wine grape skins into wine via winemaking processing.
Table 3.5 Soluble phenolic compounds of different varieties of wine grape pomace (WGP) extracted by method A and B

<table>
<thead>
<tr>
<th>WGP</th>
<th>TPC</th>
<th>RSA</th>
<th>ACY</th>
<th>TFC</th>
<th>PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg GAE/g DM</td>
<td>mg AAE /g DM</td>
<td>mg Mvd– 3 – glu /g DM</td>
<td>mg CE/g DM</td>
<td>mg/g DM</td>
</tr>
<tr>
<td>Muller Thurgau</td>
<td>15.8±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.4±0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td>25.6±1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.4±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Morio Muscat</td>
<td>11.6±1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.4±1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.870</td>
<td>20.5±3.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.9±1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>26.7±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.00</td>
<td>39.7±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.7±2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Merlot</td>
<td>25.0±3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.3±3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.060</td>
<td>40.2±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.7±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pinot Noir</td>
<td>21.4±4.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>11.2±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.010</td>
<td>32.2±4.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.1±2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

TPC, total phenolics content; RSA, DPPH radical scavenging activity; ACY, monomeric anthocyanins; TFC, total flavanol content; PAC, extractable proanthocyanidins content. Different superscripts shown within the method A column indicated the differences among WGP varieties based on LSD test (p<0.05); the comparison between method A and B employed pair t – test (p<0.05) to compare the differences in the values of the same parameter obtained by the two methods.
Table 3.6 The documentary values of total phenolic compounds from wine grape pomace (WGP), fresh wine grape berry, and other fruit byproducts

<table>
<thead>
<tr>
<th>Byproducts</th>
<th>Total phenolic content</th>
<th>Bibliography</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WGP and/or WGP skins (varieties)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RWGP, skins (Cabernet Sauvignon, Merlot and Pinot Noir)</td>
<td>21.4 to 26.7 mg GAE/g DM</td>
<td>This study</td>
</tr>
<tr>
<td>WWGP, skins (Muller Thurgau and Morio Muscat)</td>
<td>11.6 to 15.8 mg GAE/d DM</td>
<td>This study</td>
</tr>
<tr>
<td>Wine grape marc (Cabernet Sauvignon and Merlot)</td>
<td>20.2 mg GAE/g DM</td>
<td>Vatai, Škerget &amp; Kenz, 2009</td>
</tr>
<tr>
<td>RWGP (Manto Negro)</td>
<td>26.3 mg GAE/g DM</td>
<td>Llobera &amp; Cañellas, 2007</td>
</tr>
<tr>
<td>WWGP, skins (Cencibel)</td>
<td>37.6 mg GAE/g DM</td>
<td>Bravo &amp; Saura – Calixto, 1998</td>
</tr>
<tr>
<td>RWGP, skins (Airén)</td>
<td>44.8 mg GAE/g DM</td>
<td>Bravo &amp; Saura – Calixto, 1998</td>
</tr>
<tr>
<td>WWGP and WWGP skins (Roditis)</td>
<td>48.3 and 9.7 mg GAE/d DM</td>
<td>Makris, Boskou &amp; Andrikopoulos, 2007</td>
</tr>
<tr>
<td>RWGP and RWGP skins (Agiorgitiko)</td>
<td>54.0 and 36.25 mg GAE/g DM</td>
<td>Makris, Boskou &amp; Andrikopoulos, 2007</td>
</tr>
<tr>
<td>WWGP (Prensal Blanc)</td>
<td>34.9 mg GAE/g DM</td>
<td>Llobera &amp; Cañellas, 2008</td>
</tr>
<tr>
<td><strong>Fresh wine grape berry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red wine grape, skins (Pinot Noir)</td>
<td>38.4 mg/g FW *</td>
<td>Mané, et al., 2007</td>
</tr>
<tr>
<td>White wine grape, skins (Muscat of Alexandria)</td>
<td>1.2 mg GAE/g FW</td>
<td>Poudel, Tamura, Kataoka &amp; Mochioka, 2008</td>
</tr>
<tr>
<td><strong>Other fruit byproduct</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cider apple pomace</td>
<td>$6.46 \times 10^{-3}$ mg GAE/g DM</td>
<td>Suárez, et al., 2010</td>
</tr>
<tr>
<td></td>
<td>$5.5 \times 10^{-3}$ to $10.9 \times 10^{-3}$ mg GAE/g DM</td>
<td>García, Valles &amp; Lobo, 2009</td>
</tr>
<tr>
<td>Orange peel</td>
<td>2.76 mg GAE/g FW</td>
<td>Khan, et al., 2010</td>
</tr>
<tr>
<td>Acerola residues (juice production)</td>
<td>6.81 mg GAE/g DM</td>
<td>Oliveira, et al., 2009</td>
</tr>
<tr>
<td>Pineapple residues (juice production)</td>
<td>2.75 mg GAE/g DM</td>
<td>Oliveira, et al., 2009</td>
</tr>
<tr>
<td>Passion fruit residues (juice production)</td>
<td>1.03 mg GAE/g DM</td>
<td>Oliveira, et al., 2009</td>
</tr>
<tr>
<td>Strawberry residues (juice production)</td>
<td>59.77 mg GAE/g extract corresponding to the yield of extract 17.1% DM</td>
<td>Peschel, et al., 2006</td>
</tr>
<tr>
<td>Pear residues (juice production)</td>
<td>12.90 mg GAE/g extract corresponding to the yield of extract 11.4% DM</td>
<td>Peschel, et al., 2006</td>
</tr>
</tbody>
</table>
Table 3.6 (continued)

<table>
<thead>
<tr>
<th>Byproducts</th>
<th>Total phenolic content</th>
<th>Bibliography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red beet residues (juice production)</td>
<td>91.74 mg GAE/g extract corresponding to the yield of extract 20.1% DM</td>
<td>Peschel, et al., 2006</td>
</tr>
<tr>
<td>Cranberry juice pomace</td>
<td>About 11.0 mg GAE/g DM</td>
<td>Vattem, Lin &amp; Shetty, 2005</td>
</tr>
<tr>
<td>Blueberry pomace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blueberry juice pomace</td>
<td>11.9 mg GAE/g DM</td>
<td>Su &amp; Silva, 2006</td>
</tr>
<tr>
<td>Blueberry wine pomace</td>
<td>10.9 mg GAE/g DM</td>
<td>Su &amp; Silva, 2006</td>
</tr>
<tr>
<td>Blueberry vinegar pomace</td>
<td>2.3 mg GAE/g DM</td>
<td>Su &amp; Silva, 2006</td>
</tr>
</tbody>
</table>

* TPC was quantified by HPLC instead of Folin-Ciocalteu assay.

WGP: wine grape pomace; WWGP: white wine grape pomace; RWGP: red wine grape pomace; GAE: gallic acid equivalents.

Similar to the TPC values, the RSA values of RWGP were higher than those of WWGP (p<0.05). RSA of Pinot Noir was of the lowest among the RWGP, while no difference between two WWGP varieties. The TPC and RSA values were linearly correlated (R=0.989) (Figure 3.2), which was in agreement with the results by Dunonne et al. (2009).

Figure 3.2 Correlation between RSA and TPC (R=0.989), RSA and TFC (R=0.653), and RSA and PAC (R=0.667) for five WGP varieties.

Anthocyanins (ACY) was not detected in the WWGP but in the RWGP with the highest content in Merlot (1.42 mg Mal-3-glu/g DM) followed by Cabernet
Sauvignon (0.89 mg Mal-3-glu/g DM) and Pinot Noir (0.29 mg Mal-3-glu/g DM). As was stated previously, due to the thicker skins of Merlot and Cabernet Sauvignon, ACY values of those two varieties were significantly higher than that of Pinot Noir. Reported ACY values in grape pomace varied depending on the extraction and analytical methods and grape varieties. Thimothe et al. (2007) reported that ACY of Pinot Noir and Cabernet Franc was 5.9 and 67.2 mg mal-3-glu/g of lyophilized extract, respectively. Kammerer et al. (2004) quantified the total amount of ACY in 14 wine grape varieties ranged from 11.47 to 29.82 mg/g DM by using HPLC.

The total flavanol content (TFC) and proanthocyanidins (PAC) differed significantly among WGP varieties (Table 3.5). The TFC values in this study represented the amount of monomeric flavanols (free or terminal of PAC) in the aqueous solution whilst the PAC values indicated the amount of oligomeric and polymeric flavan-3–ols which were linked by C – C bonds (Muller-Harvey and McAllan, 1992), were determined by the acid butanol assay. Merlot, Muller Thurgau and Cabernet Sauvignon were of the highest content of TFC, followed by Pinot Noir and Morio Muscat (p <0.05) (Table 3.5). Likewise, Merlot possessed the highest PAC, followed by Muller Thurgau, Cabernet Sauvignon, Pinot Noir and Morio Muscat (p <0.05). Although TPC for WWGP were lower than those for RWGP, TFC and PAC values did not show significant difference between WWGP and RWGP varieties (p>0.05) (Table 3.5). Furthermore, neither linear correlation between RSA and TFC (R=0.653) nor between RSA and PAC (R=0.667) was observed (Figure 3.2), which was consistent with the previous study by Bozan et al. (2008).

Overall, WGP had high TPC values comparing with other fruit byproducts. Among the five WGP varieties evaluated in this study, Merlot ranked the first in all phenolic compounds. Muller Thurgau also displayed relatively high values of TFC and PAC.
Conclusion

This study was the first one that successfully characterized the chemical composition of polyphenols and dietary fiber of WGP skins from the Pacific Northwest. Results provided the baseline data for developing innovative utilizations of the WGP skins. Overall, the RWGP skins had high contents of TPC, RSA, ACY, TFC and PAC, making them excellent candidates for food applications such as food additives with high antioxidant capacity and food coloring ingredient. They are also good sources of dietary fiber with high contents of Klason lignin, cellulose, and hemicellulose, hence are of great potentials of being environmental-friendly supporting materials. The distinctively high amount of soluble sugar in the WWGP skins may enable them to form innovative biodegradable packaging materials with excellent flexibility. However, further study is necessary to improve WGP preparation procedures for obtaining bright color, appreciative aroma, more soluble fractions and nutrient compounds.

Acknowledgements

The author would like to thank Dr. Mark Daeschel and Dr. James Osborn for donating wine grape pomace, Dr. Seth Cohen for kindly providing wine grape skin condensed tannins, Mr. Jeff Goby for helping the analysis of neutral sugars by HPLC, and Ms. Jooyeoun Jung for analyzing resistant protein.
Reference


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CHAPTER 4

Physicochemical, Nutritional and Antimicrobial Properties of Wine Grape (cv. Merlot) Pomace Extract Based Films

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Accepted by Journal of Food Science
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Abstract

Wine grape (cv. Merlot) pomace (WGP) extract based films were studied in terms of their physicochemical, mechanical, water barrier, nutritional and antibacterial properties. Pomace extract (PE) was obtained by hot water extraction and had a total soluble solid of 3.6% and pH 3.65. Plant based polysaccharides, low methxyl pectin (LMP, 0.75% w/w), sodium alginate (SA, 0.3% w/w), or Ticafilm® (TF, 2% w/w), was added into PE for film formation, respectively. Elongation at break and tensile strength were 23% and 4.04 MPa for TF-PE film, 25% and 1.12 MPa for SA-PE film, and 9.89% and 1.56 MPa for LMP-PE film. Water vapor permeability of LMP-PE and SA-PE films was 63 and 60 g mm m\(^{-2}\) d\(^{-1}\) kPa, respectively, lower than that of TF-PE film (70 g mm m\(^{-2}\) d\(^{-1}\) kPa) (p<0.05). LMP-PE film had higher water solubility, indicated by the haze percentage of water after 24 h of film immersion (52.8%) than that of TF-PE (25.7%) and SA-PE (15.9%) films, and also had higher amount of released phenolics (96.6%) than that of TF-PE (93.8%) and SA-PE (80.5%) films. PE films showed antibacterial activity against both E. coli and L. innocua, in which approximate 5 log reductions in E. coli and 1.7-3.0 log reductions in L. innocua were observed at the end of 24 h incubation test compared with control. This study demonstrated the possibility of utilizing WGP extracts as natural, antimicrobial, and antioxidant promoting film forming material for various food applications.

Key words: wine grape pomace extract, edible films, solubiilty, total phenolic compounds, physicochemical and antibacterial property
Introduction

The increasing demand for environmentally friendly packages forced the researchers to conduct study on biodegradable and edible packaging materials. Some recently developed edible films have shown their competitive mechanical and mass transfer properties respect to the synthetic ones. In addition, the capability of carrying functional substances, such as antioxidant and antimicrobial agents has further enhanced their functionality to extent shelf-life of packaged food products (Han and Gennadios, 2005).

Fruit and fruit byproducts have attracted great interests as film forming components because of their rich contents in celluloses, hemicelluloses, pectin, pigments, flavors, and bioactive compounds, such as polyphenolics with demonstrated antioxidant and antimicrobial properties. Wine grape pomace (WGP), the by-residual from wine processing composed of a mass of skins, seeds, and stems, is a rich source of dietary fibers, and possesses significant amount of polyphenols (Katalinić et al., 2010). WGP extracts may be utilized as film forming materials since the extracts contain pectin, celluloses, and sugars (Deng et al., 2011), the essential compounds for film formation. The natural pigments, flavors, and polyphenols from WGP extracts would provide additional benefits to its applications.

Different approaches may be employed when forming films using fruit or fruit byproducts. Fruit purees, such as mango, apple, and peach puree contain high soluble polysaccharides, thus can be directly utilized as basic film forming material with the aid of plasticizer to improve film functionality (McHugh et al., 1996; McHugh and Senesi, 2000; Azerodo et al., 2009). When using fruit byproducts, such as pomace with relative low soluble polysaccharides, polysaccharides and other functional compounds from pomace are first extracted using water or other food grade solvents. The extracts are then directly incorporated into film forming solutions, freeze-dried or concentrated for later use. For this application, a small amount of film forming material (polysaccharide or protein) is usually required in order to obtaining films with desirable mechanical and barrier properties (Mayachiew and Devahastin, 2010;
Corrales et al., 2009; Hayashi et al., 2006). We have previously developed cranberry pomace based edible films by adding pectin and glycerol into the pomace extracts (Park and Zhao, 2006). The resulting films had compatible mechanical strength and water vapor permeability as other edible films, such as whey protein isolate based films (McHugh and Krochta, 1994; Shaw et al., 2002), plus an attractive bright red color and the distinctive cranberry flavor.

Different polysaccharides may be incorporated into PE to assist the film formation. Low methoxyl pectin (LMP), composed of galacturonic acid backbone chains with less than 50% of esterified carboxyl groups, is one of the non-starch polysaccharide film forming materials. It works well with other polysaccharides, such as starch and chitosan (Mariniello et al., 2003; Coffin et al., 1993; Fishman and others 1994) and plasticizers as polyvinyl alcohol, sorbitol, or glycerol (Fishman and Coffin, 1998; Park and Zhao, 2006) to obtain films with excellent mechanical properties. Sodium alginate (SA), widely used in food industry (Mancini et al., 2002), is also used as film forming material with glycerol to form films with good tensile strength and elongation (Wang et al., 2007). Cellulose gums, having a structure of D-glucose units and partial carboxymethylation of the hydroxyl groups, is water soluble and compatible with other film forming materials, such as starch, proteins, alginate, and pullulan (Zechar and Gerrish, 1997; Tong et al., 2008). Carrageenan from seaweed has also been widely utilized as gelling agent and film forming material (Thomas, 1997). In this study, three commercial polysaccharides, LMP, SA, and Ticafilm® (mixture of cellulose gum, SA, and carrageenan, TIC Gum, Belcamp, MD, USA) were incorporated into WGP extracts for forming films. All these materials are plant based, food-graded, and colorless with good film forming properties.

The objectives of this study were to investigate the feasibility of utilizing WGP water extracts with the incorporation of a small amount of commercial polysaccharides (LMP, SA and Ticafilm®) as film forming materials, and to evaluate the physicochemical, antioxidant, and antimicrobial properties of the films. Based on our previous success on developing cranberry pomace extracts based edible films
(Park and Zhao, 2006) and identified high amount of bioactive compounds in wine grape pomace (Deng et al., 2011), we hypothesized that WGP water extract can be used as a base to develop edible films with antimicrobial and antioxidant activities, and with similar mechanical and water barrier properties respect to the widely reported edible films, with the aid of additional polysaccharides. Based on our best knowledge, the polysaccharide based film literature has virtually no reports on developing edible films using WGP extracts as film forming material.

Materials and Methods

Materials

Fresh red wine grape pomace cv. Merlot (Vitis vinifera) was donated by the Oregon State University Research Winery (Corvallis, OR, USA) in the fall of 2009 and immediately stored at -24 °C till processing. Three types of commercial polysaccharides were provided by TIC Gum (Belcamp, MD, USA), including LM 32, a low methoxyl pectin (LMP) with a degree of esterification 17% to 25%; TICA-algin® HG 400, sodium alginate (SA) of medium viscosity; and Ticafilm® (TM), a mixture of sodium alginate, carrageenan, and cellulose gum. Glycerol from Fisher Scientific Inc. (Fair Lawn, NJ, USA) was employed as a plasticizer.

Folin-Ciocalteu (FC) reagent (2N) (Sigma Chemical Co., MO, USA) was used to determine total phenolic content. Plate count agar (PCA), tryptic soy agar (TSA), tryptic soy broth (TSB), and brain heart infusion broth (BHI) were purchased from Becton Dickinson and Co. (Sparks, MD, USA). Bacterial cultures, Escherichia coli ATCC 25922 (E. coli) and Listeria innocua ATCC 51742 (L. innocua) were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA).

Preparation of red wine grape pomace extracts

Only the skins of red wine grape pomace were used for the extraction. The skins were separated manually from the seeds and stems, dried at 70 °C and 10% RH for 24 h (T10RS, Tenney Environmental, Williamsport, PA, USA), and then milled to pass a 20 mesh (Thomas Scientific, Swedesboro, NJ, USA) as described by Park et al.
(2009). The dried pomace powders were packed and sealed in Ziploc® storage bags (S.C. Johnson & Son, Inc., Racine, WI, USA) and stored at -24 °C until utilization.

Pomace extract (PE) was prepared according to the procedures by Park and Zhao (2006) with some modifications (Table 4.1). Briefly, pomace powders were homogenized with 75 °C preheated deionized (DI) water at a ratio of 1:4 (w/w) at 4,000 rpm (PT 10-35, Kinematica, Littau, Switzerland) for 10 min for enhancing the release of soluble carbohydrates, polyphenolics, and pigments into the solution. The mixture was then placed into a 75 °C water bath (Precision, Winchester, VA, USA) for 30 min following by squeezing out water soluble extract through 4 layers of cheese cloths. The obtained solution was centrifuged at 10,000 g and filtrated through Whatman 1 filter paper (Whatman Inc., Piscataway, NJ, USA) for three times until crystalline salts (Jackson, 2000) were no longer observed. The filtrate was the pomace extract used for film formation.

**Table 4.1 Formulations of wine grape pomace (WGP) extract based film forming solutions (FFS)**

<table>
<thead>
<tr>
<th>Pomace extract (PE)</th>
<th>WGP powder: hot water = 1:4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polysaccharides (w/w FFS, %)</td>
</tr>
<tr>
<td><strong>Film type</strong>*</td>
<td>PE</td>
</tr>
<tr>
<td>LMP-PE</td>
<td>99.0</td>
</tr>
<tr>
<td>SA-PE</td>
<td>99.7</td>
</tr>
<tr>
<td>TF-PE</td>
<td>98.0</td>
</tr>
</tbody>
</table>

*LMP= low methoxyl pectin; SA = sodium alginate; TF = Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).

**Film formation**

Pomace water extract alone does not contain enough polysaccharides to form films with desirable mechanical and barrier properties, thereby additional polysaccharides, LMP, SA and Ticafilm®, were incorporated in the film forming solutions in order to evaluate their compatibility with PE. They were all plant based and food graded non-starch polysaccharides and are commercially available as film.
forming materials. In addition, glycerol was added as a plasticizer in some film formula to improve film flexibility.

Preliminary studies allowed finding out the best ratio of polysaccharide and PE as edible film. Our previous study (Park and Zhao, 2006) has reported that the films based on cranberry pomace extract, 0.75% LMP, and 0.25% glycerol (w/w FFS) have functional properties similar to other polysaccharide based films.

This formulation was applied for developing wine grape pomace extract based LMP-PE film. For SA films, the most appropriate concentration was 0.3% SA (w/w FFS) for forming SA-PE film with easy film casting and desirable film performance. Our preliminary tests identified that 2% Ticafilm® (w/w FFS) is necessary to form TF-PE films. Table 4.1 summarizes the final formulations of the film forming solutions.

For preparing the FFSs, accurately weighed amount of PE was first warmed up to 35-40 °C in a water bath, and then 0.75% LMP, 0.3% SA, or 2.0% Ticafilm® was slowly added with constant stirring. For LMP-PE FFS, 0.25% (w/w FFS) glycerol was also added when LMP was completely dissolved in the PE solution. The FFSs were stirred at room temperature for 1 h and further homogenized at 2,000 rpm for 60 s for preventing the lump formation. Finally, the FFSs were degassed under water aspirator vacuum until no bubble was observed. The pH values of FFSs and PE were measured before casting films.

Total soluble solid content (TSS, %) of FFS was a critical factor for obtaining uniform film thickness, and was measured by a digital refractometer (Brix RA-250 HE, Kyoto Electronics Manufacturing Co. Ltd., Kyoto, Japan). A calculated amount (Eq. 4.1) of each degassed FFS was cast onto a Teflon-coated glass plate with an area of 260 mm×260 mm.

\[
TSS_{LMP-PE} \times W_{LMP-PE} = TSS_{SA-PE} \times W_{SA-PE} = TSS_{TF-PE} \times W_{TF-PE} \quad (4.1)
\]

where \( W \) was the weight of FFS (g). The FFSs were poured onto the plates and dried at ambient conditions (24±2 °C and 40% ± 5% RH) for 36-48 h. Dried films were then peeled and conditioned inside a cabinet assembled by our lab at 25 °C and
81 ± 2% RH (provided by saturated sodium bromide) for at least 24 h before any testing. The FFSs and the films were performed in triplicate.

**Determination of film color, water activity, and moisture content**

Color of each individual film was measured at 3 random locations using a colorimeter (Lab-Scan II, HunterLab, Inc., Reston, VA, USA) which was standardized by a black and a white color plate ($X_{CIE} = 78.25$, $Y_{CIE} = 82.85$ and $Z_{CIE} = 85.83$) with illuminant D65, 0/45 geometry, and 10° observer. CIE $L^*$, $a^*$, $b^*$ values were recorded, and average values were reported for each film replication (Park and Zhao, 2006).

Water activity ($\alpha_w$) of the films was measured by using the AquaLab water activity meter (Decagon AquaLab Inc., Pullman, WA, USA). The instrument was pre-standardized using 8.57 M LiCl ($\alpha_w = 0.500$). Moisture content was determined by drying films at 75 °C in forced air convection oven (Precision Scientific, Inc., Chicago, IL, USA) for 24 h, and expressed as the percentage of weight loss after drying.

**Mechanical properties**

Six strips (25 mm x 86 mm) of each film type at each replication were used for the measurement of film thickness, film density and mechanical properties. The film thickness was measured at 5 randomly selected spots of each strip, and the average thickness was reported. Film density was obtained by the weight of each film strip divided by the volume of each strip, and the average of film density was expressed as gram per milliliter. A texture analyzer (TA XT2i, Texture Technologies Corp., Scarsdale, NY, USA) was utilized to determine mechanical properties by using modified ASTM D882 standard (ASTM Intl, 2001; Park and Zhao, 2006). Briefly, a film strip was mounted on the sample grips (TA96) between which the distance was 80 mm with the crosshead speed of 0.4 mm/s. The maximum loaded force (N) and elongation of the strip ($\Delta L$) was recorded. The tensile strength (TS) was reported as maximum loaded force (N) divided by the area of cross section (mm$^2$) and the elongation at break (EL %) was expressed as $\Delta L$ divided by the initial length of tested strip and multiplied by 100%. 
**Water vapor permeability**

Water vapor permeability (WVP) of the films was measured by using the cup method according to ASTM E 96 (ASTM Intl. 2000) at 25 °C and 100/50% RH gradient. Eleven mL of DI water was added into a Plexiglas test cup with inner depth and diameter of 15 mm and 57 mm, respectively. A film specimen (75 mm × 75 mm) was sealed on the top of the cup by vacuum grease and the distance between film and water surface was 10.7 mm. Test cup assemblies were placed in the same temperature and humidity controlled chamber conditioned at 25 °C and 50% RH. Each cup assembly was weighted every one hour for 6 h and the weight loss versus time was plotted linearly. WVP (g mm m⁻² d⁻¹ kPa⁻¹) was calculated by following the WVP correction method (Gennadios et al., 1994; Park and Zhao, 2006). WVP was determined twice for each film type at each replication.

**Microstructures**

Microstructure of the films was evaluated using a Nikon Eclipse E400 microscope (Nikon Co., Tokyo, Japan) equipped with an extended digital camera (Q imaging, Surrey, BC, Canada). The surfaces of films were observed at a magnification of 40X.

**Total phenolics content (TPC) of FFSs and phenolics release from films into aqueous solution**

To evaluate TPC of FFSs, 1 g of FFS was diluted to 10 mL with DI water in a 10 mL volumetric flask. The TPC of FFS for each film type was calculated by multiplying weight of FFS per casted film (260 mm x260 mm) and TPC per gram of FFS solution. The TPC released from the films was monitored by placing 1 g of film specimen into 100 mL DI water with constantly shaking in an environmental shaker (100 rpm) based on the method by Mayachiew and Devahastin (2010) with some modifications. A 2 mL of supernatant was obtained at 10 different sampling times between 0 to 24 h, and then stored at -20 °C until TPC analysis. The total released TPC was calculated as the released TPC accumulated after 24 h and expressed as mg
gallic acid equivalent per film (260 mm x 260 mm). TPC release test was carried out twice for each film type at each replication.

TPC was quantified by Folin-Ciocalteu (FC) assay (Singleton and Rossi, 1965). Briefly, 0.5 mL 2N FC reagent, 0.5 mL sample solution, and 7.5 mL DI water were mixed in a test tube and settled at room temperature for 10 min before 3 mL of 20% CaCO₃ was added and reacted at 40 °C for 20 min. The sample was read at 765 nm using a spectrophotometer (UV160U, Shimadzu, Kyoto, Japan).

**Water solubility**

One gram of the PE film specimen was placed into 100 mL DI water as described above. Water solubility of the films was evaluated by monitoring the changes of turbidity in the aqueous solution using a ColorQuest Sphere spectrophotometer (ColorQuest HunterLab, HunterLab, Inc., Reston, VA, USA) (0/45 geometry, 10° observer, illuminant D₆₅). The haze of solution was recorded at 0.5, 2, 4, 8, 12, and 24 h by placing the supernatant into 10 mm length quartz cell (Ngo and Zhao, 2009). The experiment was carried out once for each film type at each replication.

**Antibacterial activity against Escherichia coli and Listeria innocua**

To investigate the antibacterial activity of PE films against Gram-negative and Gram-positive bacteria, bacterial strains of *Escherichia coli* ATCC 25922 and *Listeria innocua* ATCC 51742, a modified method by Duan et al. (2008) was applied. A 0.35 g of PE film specimen was placed in a Petri dish (85 mm diameter) which contained 10 mL of bacterial culture with an initial cell concentration at approximately 10⁵ CFU/mL. Bacterial culture without film specimen was used as control. The Petri dishes were shaken at 100 rpm at room temperature, and the samples were taken at 0, 4, 8, and 24 h. The samples were diluted with phosphate-buffered saline (PBS) and plated on TSA or BHI agar for *E. coli* and *L. innocua* enumeration, respectively. Plates were incubated for 24 h at 35 °C for *E. coli* and at 30 °C for *L. innocua* before
counting the number of colonies. For each bacterial strain, each film type was tested on triplicate.

**Experimental design and statistical analysis**

Three film types (SA-PE, LMP-PE and TF-PE) were prepared independently on triplicate using a completely randomized design (CRD). For film thickness, tensile strength, elongation, and density, six pieces of each film type were measured for each replication. For color, three measurements were performed for each film type. For the TPC releasing test and WVP, two measurements were conducted for each film type at each replication. For TSS, pH, water activity, moisture content, and TPC of FFS, one measurement was conducted for each film type at each replication. Antibacterial activity was conducted on triplicate for each bacterial strain. Results were expressed as mean ± SD. One way ANOVA (Tukey-Kramer multi-comparison) was used to test the differences among three film types using SAS (SAS Inc., Cary, NC, USA) (p<0.05).

**Results and Discussion**

**Total soluble solid (TSS) and pH values of PE and FFS**

Wine grape pomace extract had a TSS of 3.6%, the addition of TF or LMP into PE significantly increased TSS of FFSs (Table 4.2), in which the TSS of TF-PE and LMP-PE FFSs was increased by about 47% and 21%, respectively (p<0.05) and the FFSs were visibly more viscous than that of PE alone. Adding 0.3% SA did not change the TSS of SA-PE FFS (p>0.05). These results were similar to the report by Park and Zhao in which adding same amount of LMP or HMP (high methoxyl pectin) into cranberry pomace extract increased the TSS to a similar level (Park and Zhao, 2006). Park and Zhao (2006) indicated that the soluble solid ratio of PE and added polysaccharides in FFS is critical for film formation and resulting film functionality due to the characteristics of the different polysaccharides. Table 4.2 shows the formulations and ratios of PE, polysaccharide and glycerol.
Table 4.2 Total soluble solids (TSS), pH and casting weight of film forming solutions (FFS)*

<table>
<thead>
<tr>
<th>FFSs+</th>
<th>TSS (%)</th>
<th>pH</th>
<th>Casting weight</th>
<th>Ratio of soluble solid in PE, added polysaccharide and glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>3.60±0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.65±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LMP-PE</td>
<td>4.37±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.65±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>226.59±3.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PE:LMP:Glycerol = 4.8:1:0.3</td>
</tr>
<tr>
<td>SA-PE</td>
<td>3.77±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.79±0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>261.65±2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>PE:SA:Glycerol = 12.0:1:0</td>
</tr>
<tr>
<td>TF-PE</td>
<td>5.30±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.11±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>188.39±3.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>PE:TF:Glycerol = 1.8:1:0</td>
</tr>
</tbody>
</table>

*Values within the same column with the different superscript meant significant difference among the films (p<0.05), n=3.

+ LMP = low methoxyl pectin; SA = sodium alginate; TF = Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).

Different polysaccharides have different gel formation mechanisms. SA and LMP require divalent cations, such as Ca<sup>2+</sup>, carrageenan needs mono- or divalent cations, while multivalent cations, such as Fe<sup>3+</sup> is necessary for cellulose gum as the crosslinking agents to link the adjacent chains to form gels (Onsøyen, 1997; May, 1997; Thomas, 1997; Zecher and Gerrish, 1997). Based on our previous study, wine grape pomace (cv. Merlot) contains different types of crosslinking agents, such as minerals, proteins, organic acids, and phenolic acids, at least 22% on dry matter (except organic acids) (Deng et al., 2011). Moreover, some of those fractions were water soluble compounds. Therefore, there were sufficient crosslinking agents in the PE to assist the film formation without the need of adding other additional crosslinking agents.

The pH of the PE was 3.65. About 90% of organic acids in grapes and wines were malic and tartaric acid (Jackson, 2000). Therefore, it could be concluded that those two organic acids were also dominated in the wine grape PE, thus resulted in the high acidity of wine grape PE. Adding TF and SA increased the pH of FFSs to 4.11 and 3.79, respectively, while the pH of LMP-PE FFS was similar to that of PE. The pH value of wine grape PE was higher than that of cranberry pomace extract (pomace: water= 1:5, pH=2.86) reported by Park and Zhao (2006).
Microstructures

Figure 4.1 shows the surface microstructures of the films under the 40× magnification. Globules were observed on the surfaces of all three types of films, in which the TF-PE film formed the largest globules followed by LMP-PE and SA-PE films. In the TF-PE films, particles from the film forming materials were un-uniformly dispersed in the film matrix, reflected by the lower density (Table 4.3) and higher water vapor permeability (Figure 4.2) of TF-PE films as reported and discussed in the later sections. Compared with TF-PE film, the LMP-PE and SA-PE films were more compact, dense and uniform, and had similar surface structure. The larger globule formation in the TF-PE films might be attributed by the remarkably higher amount of Ticafilm® material employed in the FFS resulting in decreased homogeneity of TF-PE film, as similarly observed by Kayserilioğlu et al. (2003) that the increase of xylan in wheat gluten films enlarged the globular formation and reduced the homogeneity of the films.
Figure 4.1 Surface microstructures of wine grape pomace extract based films viewed at magnification 40×; (a) TF-PE; (b) LMP-PE; (c) SA-PE. LMP=low methoxyl pectin; SA=sodium alginate; TF=Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).
Table 4.3 Moisture content, water activity, density, mechanical properties and color of wine grape pomace extract based films

<table>
<thead>
<tr>
<th>Film+</th>
<th>Weight (g)</th>
<th>Moisture content (%)</th>
<th>Water activity (g/mL)</th>
<th>Density (g/mL)</th>
<th>TS (MPa)</th>
<th>EL (%)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP-PE</td>
<td>12.0±0.6a</td>
<td>20.5±1.0a</td>
<td>0.470±0.026a</td>
<td>1.46±0.06a</td>
<td>1.56±0.39b</td>
<td>9.89±1.56b</td>
<td>18.17±1.86a</td>
<td>6.97±1.00a</td>
<td>9.70±2.32a</td>
</tr>
<tr>
<td>SA-PE</td>
<td>11.5±0.7a</td>
<td>15.5±2.3b</td>
<td>0.484±0.029a</td>
<td>1.53±0.02a</td>
<td>1.12±0.18b</td>
<td>25.07±2.94a</td>
<td>18.97±0.79a</td>
<td>6.66±0.47a</td>
<td>8.83±0.92a</td>
</tr>
<tr>
<td>TF-PE</td>
<td>11.5±0.7a</td>
<td>11.7±0.4c</td>
<td>0.470±0.035a</td>
<td>1.29±0.09b</td>
<td>4.04±0.29a</td>
<td>23.05±2.82a</td>
<td>21.40±1.43a</td>
<td>7.07±0.58a</td>
<td>9.80±1.25a</td>
</tr>
</tbody>
</table>

*Values within the same columns with the different superscript meant significant difference among the films (p<0.05).
+LMP = low methoxyl pectin; SA = sodium alginate; TF = Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum)
Physicochemical properties

Depending on the film formulation, it took 36 to 48 h to dry the films at room condition, of which the TF-PE films dried the fastest (36 h), probably due to the lowest amount of casting solutions used, followed by LMP-PE and SA-PE films. Moisture contents (MC) of the films differed significantly (p<0.05), 20%, 16%, and 12% for LMP-PE, SA-PE, and TF-PE films, respectively (Table 4.3). LMP-PE film possessed the highest MC, probably due to the addition of glycerol (0.25% w/w) that is known for its water attracting and holding capacity. There was no difference in water activity (α_w) among the different films, 0.470 to 0.484 (Table 4.3). The low α_w values below 0.60 proved that the films developed in this study were stable and capable of retarding microbial proliferation under room conditions (Fontana and Campbell 2004). TF-PE film had the lowest density compared with other two films, which agreed with the observed loose surface microstructures of TF-PE films (Figure 4.1).

Color of the three types of films was not different (p>0.05) (Table 4.3). L* values (lightness) were in a range of 18.2 to 21.4, a* values (redness) of 6.7 to 7.1, and b* values (yellowness) of 8.8 to 9.8. Anthocyanins in the PE contributed to the color of the films. Anthocyanins are pH sensitive, showing different colors depending on the pH values. In this study, pH values of the FFSs were in the range of 3.65 to 4.11 (Table 4.2) within which the FFSs were in similarly red color. After drying, films retained visually red. The polysaccharides (LMP, SA, and TF) and glycerol added into FFSs were colorless, thus no impact on the color of the films. This observation was consistent with our previous findings on the pectin-cranberry pomace extract based films (Park and Zhao, 2006).

Mechanical properties

Tensile strength (TS) were significantly different among the three film formulations (p<0.05) (Table 4.3), in which TS of TF-PE film was 4.0 MPa, about 2.6 times higher than those of other two films. Elongation at break (EL%) of SA-PE and
TF-PE films were 153% and 133% that of LMP-PE film. The type and concentration of polysaccharides used for forming films significantly affected film mechanical properties: the SA-PE film was the mostly elastic, but the weakest in strength, while the LMP-PE film was weak in both elasticity and tensile strength. On the other hand, TF-PE film had the best mechanical properties, strongest and most flexible. Ticafilm®, a mixture of cellulose gum, carrageenan, and sodium alginate (portion for each ingredient is unavailable), is claimed to be of superior film forming properties. As indicated before, our preliminary study found that a high amount of Ticafilm® (2%, w/w FFS) was necessary to carry the soluble solids from PE for forming the TF-PE film with smooth surface and sufficient tensile strength. With the increased amount of Ticafilm® material, TS was largely increased without reducing EL%. This result agreed with the report by Azeredo et al. (2009) in which increased loading of cellulose nanofibers into mango puree based edible films strengthened TS without diminished the elastic properties unless the loading cellulose nanofibers amount was over the optimum amount.

PE contains cellulose, hemicellulose, pectic polysaccharides, minerals, proteins, lipids, and organic acids (Deng et al., 2011). The polysaccharides (cellulose gum, carrageenan, sodium alginate, and low methoxyl pectin) added into PE have different functional groups, including hydroxyl, sulphate (specific to carrageenan), carboxyl, ester, and acid groups. Various interactions might occur during the film forming process. Unfortunately, it was impossible to conclude the effects of these interactions on the mechanical properties of the films since the specific proportion of each compound in the FFS was unknown.

Compared with other fruit byproduct based edible films (Table 4.4), TF-PE film could be considered as strong and flexible, SA-PE film was soft and flexible, but LMP-PE film was not competitive with other films in terms of the mechanical properties.
<table>
<thead>
<tr>
<th>Film forming material</th>
<th>TS (MPa)</th>
<th>EL (%)</th>
<th>WVP (g mm m$^{-2}$ d$^{-1}$ kPa$^{-1}$)</th>
<th>Condition for WVP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranberry pomace extract (1.38% TSS) incorporating with 0.50-0.75% of pectin (LMP and HMP) and 0.25% of plasticizer (sorbitol or glycerol)</td>
<td>1.0-8.1</td>
<td>12.9-47.7</td>
<td>70-80</td>
<td>25 °C; 100%/50% RH</td>
<td>Park and Zhao (2006)</td>
</tr>
<tr>
<td>Spinach extract (0-1.05% TSS) and wine grape pomace extract (cv. Merlot) (0-8.16% TSS) incorporating with cassava starch (5%), sucrose (0.7%) and inverted sugar (1.4%)</td>
<td>1.8-4.2</td>
<td>65-217</td>
<td>1406.0-8804.75</td>
<td>75%/0% RH</td>
<td>Hayashi and others (2006)</td>
</tr>
<tr>
<td>26% of apple puree (38% TSS), 70.5% of 3% LMP solution, 0.25% of ascorbic acid and citric acid, 3% of glycerol and 0-1.5% of carvacrol (w/w)</td>
<td>1.25-1.93</td>
<td>38.1-50.3</td>
<td>91.4-121.2</td>
<td>25±1 °C; 77.4-80.4%/0 RH</td>
<td>Du and others (2008, a)</td>
</tr>
<tr>
<td>26% of apple puree (38% TSS), 71.5% of 2% alginate solution, 1.0% of 1% N-acetylcysteine and 1.5% glycerol and 0-0.5% essential oils</td>
<td>2.47-2.90</td>
<td>51.1-58.3</td>
<td>104.9-126.0</td>
<td>25±1 °C; 63.5-67.1%/0 RH</td>
<td>Rojas-Graü and others (2007)</td>
</tr>
<tr>
<td>0-36 g of cellulose nanofibers and 100 g of mango puree solution (27.5% TSS)</td>
<td>4.09-8.76</td>
<td>31.5-43.3</td>
<td>40.1-63.8</td>
<td>N/A</td>
<td>Azeredo and others (2009)</td>
</tr>
<tr>
<td>50.0-90.0% of PVA (10% TSS), 10.0-50.0% of natural fibers (orange byproducts, apple pomace or sugar cane bagasse) and various amounts of glycerol, urea, starch, hexamethoxymelamine and citric acid</td>
<td>2-52</td>
<td>5.0-20.0</td>
<td>N/A</td>
<td>N/A</td>
<td>Chiellini and others (2001)</td>
</tr>
</tbody>
</table>
### Table 4.4 (continued)

<table>
<thead>
<tr>
<th>Film forming material</th>
<th>TS (MPa)</th>
<th>EL (%)</th>
<th>WVP (g mm m(^{-2}) d(^{-1}) kPa(^{-1}))</th>
<th>Condition for WVP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango puree (19% TSS)</td>
<td>1.2</td>
<td>18.5</td>
<td>213.2</td>
<td>27°C, 50%/0 RH</td>
<td>Sothornvit and Rodsamran (2008)</td>
</tr>
<tr>
<td>Small amount of cavacrol and tomato paste which was formed by mixing 30% of tomato puree (31% TSS) and 70% of 3% high methoxyl pectin solution together</td>
<td>8.9-14.8</td>
<td>6.0-11.6</td>
<td>50.64-64.32</td>
<td>25±1 °C; 81.5-85.1%/0 RH</td>
<td>Du and others (2008, b)</td>
</tr>
<tr>
<td>4% of dry banana starch (native or oxidized), 2% of glycerol and 0.2% of sunflower oil</td>
<td>3.5-4.0</td>
<td>12.5-35.0</td>
<td>17.3-121.0</td>
<td>25°C; 100%/57% RH</td>
<td>Zamudio-Flores and others (2006)</td>
</tr>
</tbody>
</table>
Water vapor permeability

Thickness of the films directly influences WVP values. The thickness of TF-PE film was about 160 μm, about 20 μm and 30 μm higher than that of MP-PE and SA-PE films, respectively (Figure 4.1). WVP of TF-PE, LMP-PE and SA-PE films were about 70, 63, and 60 g mm m⁻² d⁻¹ kPa at 25 °C and 100/50% RH, respectively, with TF-PE film had significantly higher WVP value than that of other films (p<0.05) (Figure 4.2). All polysaccharides evaluated in this study are hydrophilic materials with high water holding capacities, thus poor water barrier properties when forming films. The larger thickness of TF-PE film may contribute to its high WVP value compared with the other two films as previous study indicated that the thicker the hydrophilic edible film, the higher the WVP values (McHugh et al., 1993). Also, the less compacted structure of TF-PE film might reduce its moisture barrier ability (Figure 4.1).

![Figure 4.2 Water vapor permeability (g mm m⁻² d⁻¹ kPa⁻¹) of wine grape pomace extract based films corresponding to film thickness (μm). Different letters indicated the significant difference at p<0.05. LMP=low methoxyl pectin; SA=sodium alginate; TF=Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).](image-url)
Compared with the WVP values in other fruit (puree) and fruit byproduct based films as shown in Table 4.4, WVPs of the films from this study were relatively low. In general, fruit and fruit byproduct base edible films displayed poor moisture barrier ability owing to the hydrophilic nature of the materials unless hydrophobic materials were incorporated. For instant, WVP of apple puree based edible films ranged from 91.4-126.0 g mm m$^{-2}$ d$^{-1}$ kPa (Du et al., 2008a; Rojas-Graü et al., 2007), and mango puree base edible films with or without other film forming materials were 40.1-63.8 (Azeredo et al., 2009) or 213.2 g mm m$^{-2}$ d$^{-1}$ kPa (Sothornvit and Rodsamran, 2008). Therefore, the moisture barrier properties of wine grape PE based films from this study were promising (Figure 4.2).

**Water solubility**

Distinct differences of film solubility were observed among the three types of films (Figure 4.3). LMP-PE film was highly water soluble with the haze percentage value of its aqueous solution reached to about 46% at the end of 12 h, and the film specimens were visually disintegrated. However, the solubility significantly slowed down during the last 12 h. The haze percentage value at the end of 24 h was about 52% (Figure 4.3). Adversely, the haze percentage values of SA-PE and TF-PE films maintained stable within the first 12 h immersion in water (~7% at 12 h), and then increased to 17% and 26%, respectively at the end of 24 h, at which the more turbid aqueous solutions were observed. Compared with LMP-PE film, TF-PE and SA-PE films retained their integrity after immersion into water for 24 h.
Figure 4.3 Solubility of wine grape pomace extract based films in aqueous solution. The results are the mean of triplicates with standard deviations. LMP=low methoxyl pectin; SA=sodium alginate; TF=Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).

It has been well recognized that film solubility largely depends on the amount of crosslinking materials participating in the film forming process (Jin et al., 2004; Cao et al., 2007). In general, the more the crosslinking materials, the lower the solubility of the films. Wine grape PE provided several types of crosslinking materials, such as minerals (Ca\(^{2+}\), Mg\(^{2+}\), and Fe\(^{3+}\)) and acids (organic acids, amino acids, and phenolic acids) (Pavlath et al., 1999; Cao et al., 2007). In this study, SA-PE film had the highest ratio of total solids from PE, followed by LMP-PE and then TF-PE film (Table 4.2). The high water solubility in LMP-PE film might attribute to its high moisture content and the added glycerol which is highly water soluble. The different film forming properties of LMP (0.75% w/w FFS) and Ticafilm® (2% w/w FFS) might also impact the water soluble property of the films.

**Total phenolics content (TPC) of FFSs and TPC release from films**

The TPC values significantly differed among the three FFSs \((p<0.05)\), in the order of SA-PE (167.8 mg GAE/g FFS)> LMP-PE (148.5 mg GAE/g FFS) >TF-PE (111.5 mg GAE/g FFS) (Figure 4.4). Unlikely, the amount of TPC released from the films into distilled water at the end of 24 h in room temperature followed different orders, in which the largest amount was from LMP-PE film (144.1 mg GAE/film).
followed by SA-PE film (134.3 mg GAE/film) and TF-PE film (104.7 mg GAE/film) (Figure 4.4). About 96.6% TPC was released from LMP-PE film while 93.8% was released from TF-PE film and 80.5% from SA-PE film. Different interaction mechanisms between phenolic compounds and polysaccharides might attribute to the diverse release characterization of TPC from different films. First, the formation of polysaccharide-phenol complex might be due to the interaction of hydrogen bonds of phenolic hydroxyl groups with the oxygen atoms of the crosslinking ether bonds of sugars by which the phenolic compounds are entrapped into the polysaccharide pores (Pinelo et al., 2006). Secondly, the form of hydrophobic regions of polysaccharides might encapsulate phenolic acids inside the hydrophobic regions thereby caused the aggregation of phenolic compounds (Pinelo et al., 2006). Further, film solubility also correlated with phenolic compound released from hydrophilic films (Mayachiew and Devahastin, 2010). LMP-PE film with the highest film solubility had the highest released TPC while SA-PE films with the lowest water solubility had the lowest amount of released TPC.

![Figure 4.4](image)

**Figure 4.4** Total phenolics content (TPC) in film forming solutions (FFS) and amount of TPC released from films at the end of 24 h water immersion test. The different letters indicated the significant differences among the films at \( p < 0.05 \). LMP=low methoxyl pectin; SA=sodium alginate; TF=Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).
The release kinetics of phenolic compounds from the films is shown in Figure 4.5. All films showed almost the same rapid release rate in the first hour with the percentage TPC release reached to 69.5%, 68.7%, and 62.6% for LMP-PE, SA-PE, and TF-PE film, respectively. After the first hour, the release of TPC slowed down significantly, especially for the SA-PE film. After 4 h, almost no change in TPC release rate was observed in SA-PE film, but TPC release increased about 10% for LMP-PE and TF-PE films between 8-24 h. At the end of 24 h, TPC release was 96.6%, 93.8%, and 80.5% for LMP-PE, TF-PE and SA-PE film, respectively.

Figure 4.5 TPC releasing characterization of wine grape pomace extract based films in distilled water corresponding to time at room temperature. The results are the mean of triplicates with standard deviations. LMP=low methoxyl pectin; SA=sodium alginate; TF=Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).
No previous study had simultaneously evaluated the film water solubility and TPC release kinetics. In this study, TPC release and film solubility were not in synchronization at the first 8 h of water immersion test, where the phenolic compounds were rapidly released from the film matrix, but only LMP-PE film was highly dissolved into water at the same time period (Figures 4.3 and 4.5). The initial fast release of TPC was possibly caused by the quick dissolve of phenolic compounds which were hydrophilic, bonded with film polysaccharides, and distributed on the surface of the films. Along with the longer water immersion, the film structure relaxed and the film matrix swollen, leading to slower release of phenolic compounds (Wu et al., 2005). This might explain continuously increased TPC release from LMP-PE film matrix while TPC release from SA-PE and TF-PE films tended to be stable from 8 to 24 h.

Several previous studies have evaluated the antioxidant properties of plant extract incorporated films. Corrales et al. (2009) reported that the pea starch based films incorporated with grape seed extract (lyophilized powder) showed a low TPC migration (8%). Mayachiew and Devahastin (2010) added gooseberry concentrate into chitosan based films and reported about 70 to 90% of TPC release from the films. The TPC release from this study was comparable with the results from Mayachiew and Devahastin (2010).

**Antibacterial activity**

Overall, all types of PE films significantly delayed the bacterial growth during 24 h of growth test (Figure 4.6). At the end of 24 h, about 2 log reduction in *L. innocua* and ~5 log reduction in *E. coli* were observed compared with control (culture without added PE film). Within the same bacteria strain, three film types showed similar inhibition against the growth of *L. innocua* or *E. coli*, while the PE based films performed differently against the growth of the two different bacteria. No growth in *E. coli* cultures containing any PE film was observed during 24 h test, and a 4.9 log (TF-PE film)-5.5 log (SA-PE film) reductions were achieved compared with control. While for *L. innocua*, continuous growth during the 24 h test period was observed, but was
significantly slower than that of control (p<0.05). A 0.5-1.3 log reduction was observed at the first 4 h, followed by 2.4-3.0 log reduction at 8 h, and at 1.7-3.0 log reduction at 24 h compared with control. Hence, it could be concluded that all types of PE films inhibited the growth of E. coli and L. innocua, but E. coli was more susceptible to the PE films than that of L. innocua in this study.

WGP skins are rich in polyphenols, such as tannins, phenolic acids, and flavanols, which are proven having antimicrobial functions through different mechanisms including membrane disruption, bind to proteins, and formation of a complex with bacteria cell wall (Cowan, 1999). In addition, WGP is abundant in organic acids which have antibacterial activity against both Gram-negative and Gram-positive bacteria (Daglia et al., 2007; Kim et al., 2008). In this study, the released TPC and organic acids from the PE films may both play the roles as antibacterial agents. However, the literature contains controversial reports on the efficacy of WGP against Gram-positive or Gram-negative bacteria. Kataliníc (2010) reported that WGP skin extracts inhibited the growth of both Gram-negative and Gram-positive microorganisms. On the other hand, Özkan et al. (2004) found that S. aureus (Gram-positive) were more susceptible to grape pomace extracts (2.5%) than E. coli O157:H7 (Gram-negative bacteria) at the same concentration level. Corrales et al. (2009) indicated that grape seed extract incorporated pea starch films were able to inhibit Gram-positive, but not Gram-negative bacteria. This study demonstrated that the PE films inhibited both Gram-positive and Gram-negative bacteria, but were more effectively against Gram-negative bacteria (E. coli) than Gram-negative bacteria (L. innocua).
Figure 4.6 The inhibiting activity of wine grape pomace extract based films against *E. coli* and *L. innocua* corresponding to time at room temperature. The results are the mean of triplicates with standard deviations. LMP=low methoxyl pectin; SA=sodium alginate; TF=Ticafilm® (mixture of sodium alginate, carrageenan and cellulose gum).
Conclusion

Wine grape pomace extract based edible films with the addition of small amount of polysaccharides showed attractive color and comparable mechanical and water barrier properties to other edible films. All films had relatively low WVP. TF-PE films had high tensile strength, and SA-PE and TF-PE films showed large elongation. Hence, they may be used as colorful food wraps. The controlled release of phenolics from the film matrix illustrated their potential antioxidant and antimicrobial functions. LMP-PE film had high water solubility and fast phenolics release rate, making it a good candidate as the oral fast dissolving film in pharmaceutical or other similar applications.

Several respects are worthy further studies. First, it is necessary to enhance the water extraction of bioactive compounds and soluble polysaccharides from WGP in order to promoting the antioxidant capacity and antimicrobial functionality, meanwhile reducing the usage of extra sources of polysaccharides. Further, film forming formulations can be improved with the addition of other functional substances to strengthen the film mechanical and water barrier properties for more wide applications. Moreover, applications of wine grape pomace extract based edible films and coating should be studied on real food systems for their antioxidant and antimicrobial functions.

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CHAPTER 5

General Conclusion

This project was the first study that analyzed the chemical composition of wine grape pomace (WGP) skins grown in the Pacific Northwest of the United States, and used WGP extract to form edible films.

The obtained chemical composition data of WGP skins provide the guidance for developing their innovating applications. The high contents of total phenolic compounds (TPC) (21.4-26.7 mg gallic acid equivalent/g DM), antiradical scavenging activity (RSA) (32.2-39.7 mg ascorbic acid equivalent/g DM), anthocyanins (ACY) (0.29-0.89 mg malvidin-3-glucoside equivalent/g DM), total flavanol contents (TFC) (42.6-61.2 mg catechin equivalent/g DM) and proanthocyanidins (PAC) (11.9-24.1 mg condensed tannin/g DM) in red WGP (RWGP) skins make them excellent candidates as food additives which provides food commodities (i.e. beverages, dairy products, candies) with high antioxidant activity and bright red colors. RWGP skins are also good sources of dietary fiber (54.7-61.2% DM) with high contents of Klason lignin (32.0-41.0% DM), cellulose, and hemicellulose, hence are of great potentials of being biodegradable packaging containers. The high contents of dietary fiber and polyphenolic compounds also enable RWGP skins powder for direct usage in foods. For instant, RWGP skins powder can be a substitute of bakery flour, and it also can be added to meat products to prevent lipid oxidation and fortify them with dietary fiber. On the other hand, the distinctively high amount of soluble sugar in the white WGP (WWGP) skins (55.8-77.5% DM) may also make them form packaging materials with excellent flexibility.

WGP skins (cv. Merlot) were employed to formulate edible films with the addition of a small amount of polysaccharides. Pomace extract (PE) edible films
showed attractive color and comparable mechanical and water barrier properties to other edible films. Hence, they may be used as colorful food wraps. The low methoxyl pectin-PE (LMP-PE) film had high water solubility and fast phenolics release rate, making it a good candidate as the oral fast dissolving film in pharmaceutical or other similar applications. All PE based edible films were able to release phenolics and prevent the growth of *Escherichia coli* and *Listeria innocua* in an aqueous system, demonstrated their potentials as functional edible films with antioxidant capacity and antimicrobial activity.

Several respects of this project, however, are worthy further studies. First, further study is necessary to improve WGP preparation procedures for obtaining bright color, appreciative aroma, more soluble fractions and nutrient compounds. Secondly, more food applications of WGP skins can be developed based on the chemical composition data from this project. Thirdly, further study should be addressed to improve mechanical and mass barrier properties of PE films by incorporating with other film forming materials, to enhance the antioxidant capacity and antimicrobial activity by optimizing the polyphenolic recovery of extract from WGP skins, and to apply PE based edible films on real foodstuffs.
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APPENDIX

Chromatograms and correction factors of neutral sugars

Neutral sugars (NS) were the monosaccharides from acid hydrolysis of the structural carbohydrates (dietary fiber). NS of WGP were characterized by eluting through Aminex HPX–87P column equipped in HPLC-ELSD. The chromatograms of IDF and SDF in Pinot Noir are shown in Figure A.1 as the examples. Retention time for sugar standards were 12.567 min for glucose, 13.604 min for xylose, 14.343 min for glucose, 15.481 min for arabiose, and 15.983 min for mannose, respectively.

The effect of sulfuric acid hydrolysis on the changes of responding signals of five sugars are reported in Table A.1. Acid hydrolysis increased the responding signals in ELSD of five neutral sugars by 9% to 72%, possibly due to the water molecules trapped in the CaCO₃ precipitates when calcium carbonate neutralized the acid solution, thereby increased the concentrations of sugars in aqueous system. Therefore, the correction factors were taken into account in order to obtain accurate amount of NS in the WGP. The real concentration of each neutral in WGP was obtained by multiplying detected response of each neutral sugar by the corresponding correction factor.
a. HPLC – ELSD chromatogram of neutral sugar of IDF in Pinot Noir

b. HPLC – ELSD chromatogram of neutral sugar of SDF in Pinot Noir

c. HPLC – ELSD chromatogram of neutral sugar of SDF in Pinot Noir

Figure A.1 Neutral sugar of IDF and SDF in Pinot Noir analyzed by HPLC-ELSD

Table A.1 Correction factors of each hydrolysis neutral sugar standard in IDF and SDF after acid hydrolysis (AH)
<table>
<thead>
<tr>
<th>Glucose</th>
<th>Xylose</th>
<th>Galactose</th>
<th>Arabinose</th>
<th>Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IDF</td>
<td>SDF</td>
<td>IDF</td>
<td>SDF</td>
</tr>
<tr>
<td>Original conc.*</td>
<td>38.91</td>
<td>2.38</td>
<td>4.80</td>
<td>0.47</td>
</tr>
<tr>
<td>Conc.+ (after AH)</td>
<td>41.77</td>
<td>3.24</td>
<td>6.29</td>
<td>0.81</td>
</tr>
<tr>
<td>Correction factor++</td>
<td>0.93</td>
<td>0.73</td>
<td>0.76</td>
<td>0.58</td>
</tr>
</tbody>
</table>

*Original concentration for acid hydrolysis.
+ Detected concentration after acid hydrolysis.
++ Correction factor was equal to original concentration divided by detected concentration after acid hydrolysis.