AN ABSTRACT OF THE THESIS OF

Angela Y. Tseng for the degree of Master of Science in Food Science and Technology, Oregon State University presented on December 18, 2012.

Title: Development of Antioxidant Dietary Fibers from Wine Grape Pomace and Their Applications as Functional Food Ingredients.

Abstract approved:

Yanyun Zhao

Wine grape pomace (WGP), the byproduct from winemaking, is a good source of polyphenols and dietary fibers, and may be utilized as antioxidant dietary fibers (ADF) for food applications. The objectives of this thesis research were to first determine the phenolic compounds, antioxidant and antimicrobial activities in red WGP under different drying processes for long-term storage, and to further evaluate the feasibility of using WGP as a functional food ingredient in yogurt and salad dressing for enhancing the nutritional value and improving storability of the products.

Two types of WGP samples, pomace containing seeds and skins (P) and pomace with skins only (S) from Pinot Noir (PN) and Merlot (M) were studied. Samples were subjected to four different drying conditions: 40 °C conventional and vacuum oven, 25 °C ambient air and freeze dry. Total phenolic content (TPC, by Folin-Ciocalteu assay), anthocyanins (ACY, by pH differential method) and flavanols content (TFC, by vanillin

assay) of the samples along with their antioxidant activity (DPPH radical scavenge method, RSA) and antibacterial activity (minimum inhibition concentration, MIC) were determined during 16 weeks of storage under vacuum condition at 15±2 °C. Meanwhile, dietary fiber profile was evaluated by using gravimetric-enzyme method. Results showed that dietary fiber contents of PN-P, PN-S, M-P and M-S were 57-63% d.m. with the majority of insoluble fraction. Freeze dried WGP retained the highest bioactive compounds with TPC 21.19-67.74 mg GAE/g d.m., ACY of 0.35-0.76 mg Mal-3-glu/g d.m., TFC of 30.16-106.61 mg CE/g d.m. and RSA of 22.01-37.46 mg AAE/g d.m., followed with ambient air dried samples. Overall, TPC, TFC and RSA were higher in PN than in M, and higher in pomace than in skins, while reverse results were observed in ACY. All samples lost significant amount of bioactive compounds during storage, in which ambient air and freeze dried samples had TPC reduction of 32-56% and 35-58%, respectively at the end of 16 weeks of storage. RSA in PN-P and M-P remained more than 50 mg TE/g d.m., meaning WGP still met the criteria of ADF definition after 16 weeks of storage. WGP extracts showed higher antibacterial efficiency against *L. innocua* than that of *E. coli* with MIC of 2, 7, 3 and 8% against *L. innocua*, and 3, 6, 4 and 9% against *E. coli* for PN-P, PN-S, M-P and M-S samples, respectively. This study demonstrated that Pinot Noir and Merlot pomace are good sources of ADF even after 16 weeks of storage at 15 °C and vacuum condition.

Due to the highest antioxidant activity (RSA 37.46 mg AAE/g) and dietary fiber content (61%), PN-P was selected as ADF to be fortified in yogurt and salad dressing. Three types of WGP: whole powder (WP), liquid extract (LE) and freeze dried extract (FDE) with different concentrations were incorporated into yogurt (Y), Italian (I) and Thousand Island (T) salad dressings. TPC, RSA and dietary fiber content, major quality attributes including pH and peroxide value (PV) during the shelf life and consumer acceptance of fortified products were evaluated. The highest ADF were obtained in 3% WP-Y, 1% WP-I and 2% WP-T samples with the dietary fiber contents of 1.98%, 2.12% and 1.83% and RSA of 935.78, 585.60 and 706.67 mg AAE/kg, respectively. WP fortified products had more dietary fiber content than that of LE and FDE fortified ones because of the insoluble fractions. The pH dropped from 4.52 to 4.32 for 3% WP-Y

during three weeks of storage at 4 °C, but remained stable in WGP-I and WGP-T samples after four weeks of storage at 4 °C. Adding WGP resulted in 35-65% reduction of PV in all samples compared to the control. In WGP-Y, the viscosity increased, but syneresis and lactic acid percentage were stable during storage. The 1%WP-Y, 0.5%WP-I and 1%WP-T samples were mostly liked by consumers. Study demonstrated that WGP can be used as a functional food ingredient for enhancing nutraceutical content and extending shelf-life of the food products.

This study provided important information about the economically feasible drying methods for retaining the bioactive compounds in WGP during processing and storage and also suggested that WGP can be utilized as antioxidant dietary fiber to be fortified in consumer products to promote nutritional benefit and extend product shelf-life.

Development of Antioxidant Dietary Fibers from Wine Grape Pomace and Their Applications as Functional Food Ingredients

by

Angela Y. Tseng

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APPROVED:

Major Professor, representing 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.

Angela Y. Tseng, Author

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This dissertation is dedicated to my dearest brother, mom and dad. I am blessed that have you always stand by me with love and faith. Your philosophy and value benefit me for lifelong time and I believe we will go through challenges together. Life is full of surprises after all!

TABLE OF CONTENTS

TABLE OF CONTENTS (CON'T.)

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. INTRODUCTION

Wine grape pomace (WGP), the byproduct in winery industry after winemaking, is a rich source of polyphenols and dietary fibers (Llobera & Cañellas, 2007). The major phenolic compounds in WGP has been identified as monomeric phenolic compounds such as (+)-catechins, (−)-epicatechin and dimeric, trimeric and tetrameric procyanidins in WGP seeds (Saito, Hosoyama, Ariga, Kataoka, & Yamaji, 1998), as well as anthocyanins (mainly malvidin 3-O-glucoside), hydroxycinnamic acids, and flavonol glycosides in red WGP skins (Schieber, Kammerer, Claus, & Carle, 2004). These bioactive compounds contribute to not only the antioxidant activity by donating the hydrogen atom to the unpaired radicals, but also the antimicrobial activity against bacteria, fungus and virus (Jayaprakasha, Selvi, & Sakariah, 2003; Özkan, Sagdiç, Göktürk Baydar, & Kurumahmutoglu, 2004; Thimothe, Bonsi, Padilla-Zakour, & Koo, 2007). Meanwhile, WGP contains promising amount of dietary fiber that may provide the health benefit for controlling diabetes and obesity and reducing the risk of stroke, hypertension, coronary heart and gastrointestinal diseases (Anderson et al., 2009; Deng, Penner, & Zhao, 2011).

The term "antioxidant dietary fiber (ADF)" was first proposed by Saura-Calixto (1998). Based on the definition, ADF from fruits and vegetables should have more than 50% of dietary fiber and at least 50 mg vitamin E equivalent per gram of DPPH free radical scavenging capacity. Above characteristics should be instinct, derived from the plant properties. Therefore, the first objectives in this study was to characterize the phenolic content and chemical composition of WGP from two predominate red wine varieties in Oregon, *vinifera L. cv* Pinot Noir and *cv.* Merlot to determine whether they meet the criteria of ADF.

Dehydration of fresh WGP is usually the first step before developing further applications since fresh WGP spoils at high moisture content. However, bioactive in WGP compounds are sensitive to heat and oxygen, and may be destroyed during processing and storage. Previous studies have evaluated the different extraction methods of the phenolic compounds (Deng et al., 2011; Spigno & De Faveri, 2007), and the stability of polyphenols from fruit pomace under different water activity conditions

(Hatzidimitriou, Nenadis, & Tsimidou, 2007). Few studies have investigated how drying methods affact the polyphenols retention and their stability during long term storage. Therefore, another aim of this study was to determine the impact of four different economic drying methods (oven drying at 40 °C, vacuum drying at 40 °C, ambient air at 25 °C and freeze drying) and vacuum storage at 15 °C on phenolic compounds (total phenolic, flavonol and anthocyanin contents), antioxidant (DPPH radical scavenging) and antibacterial (minimum inhibition concentration against *E. coli* and *L. innocua*) activities of dried WGP.

WGP has been suggested as functional food ingredient to be fortified in consumer food products for enhancing nutritional and other functional properties due to their rich amount of dietary fibers and polyphenols. WGP as a good source of dietary fiber had been mixed with flour to make sourdough for rye bread (Mildner-Szkudlarz, Zawirska-Wojtasiak, Szwengiel, & Pacyński, 2011), cereal bars, pancakes and noodles (Rosales Soto, Brown, & Ross, 2012). WGP has also been incorporated with corn chips (Rababah et al., 2011), minced fish (Sanchez-Alonso, Jimenez-Escrig, Saura-Calixto, & Borderias, 2008) and chicken patties (Sáyago-Ayerdi, Brenes, & Goñi, 2009) as a natural antioxidant to prevent lipid oxidation.

Functional foods represent an important, innovative and rapidly growing part of the overall food market. Yogurt is the most popular fermented dairy product with high nutritional value, but not considered a significant source of polyphenols and dietary fibers. Different sources of dietary fibers from fruit and fruit extract have been fortified into yogurt to determine the rheological properties and stability of physicochemical properties (Karaaslan, Ozden, Vardin, & Turkoglu, 2011; Sendra et al., 2010; Staffolo, Bertola, Martino, & Bevilacqua, 2004). On the other hand, salad dressing with high amount of fat content can be readily oxidized, led to the formation of undesirable volatile compounds during processing and storage (Min & Tickner, 1982). Natural antioxidants, such as honey and orange pulp, have been added into salad dressing in order to prevent oxidative deterioration of unsaturated fatty acids (Chatsisvili, Amvrosiadis, & Kiosseoglou, 2012; Rasmussen et al., 2008). As a result, the last objective of this study was to investigate the feasibility of fortifying WGP in yogurt and salad dressing to extend enhance nutraceutical benefit and extend shelf-life of the products.

In summary, there were three specific research objectives in this study: 1) to determine the phenolic compounds and dietary fiber content to confirm if WGP meet the ADF criteria; 2) to investigate the effects of different drying methods and storage time on the retention and stability of phenolic compounds, antioxidant and antibacterial activities of WGP; 3) to evaluate the feasibility of using WGP as functional food ingredient for enhancing the nutritional value and improving the storability of yogurt and salad dressings.

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CHAPTER 2. LITERATURE REVIEW

2-1. Wine grape pomace

2-1-1. Red wine grape pomace

The United States is the 4th largest wine producing country in the world. According to USDA statistic, 4.142 million tons of grapes were wine grapes applied for winemaking in 2011, but only 0.403 million tons of grapes were processed for other products, such as juice, jams and jellies [1]. Oregon is one of the predominate wine grape production states in the US northwest pacific region, and the production increased about 40%, from 19,753 tons in 2010 to 27,667 tons in 2011. Based on the USDA-NASS record [2], the production of red wine grapes is higher than the white ones in Oregon with the most popular varieties of Pinot Noir (23,726 tons), Syrah (1,319 tons), Cabernet Sauvignon (1,206 tons), Merlot (1,129 tons) and Cabernet Franc (287 tons).

With the concept of the "French paradox" first brought out by Sumuel Black in 1819, numerous studies have investigated the bioactive compounds in red wine associated with the reduction on risk of coronary heart disease. Red wine processing involves crushing or pressing whole grapes in order to release the juice and extracts the nutrients and polyphenols. Unlike white wine used grape juice ferments within short maceration (couple hours), red wine process includes grapes skins, seeds and stems fermenting with prolonged maceration up to 3-5 days. During fermentation, sugars in the wine must are converted to ethanol by yeast (usually *Saccharomyces cerevisiae* and *Leuconostocoenos*) under 24-27 °C. Meanwhile, fermentation also promotes the extraction of anthocyanin and tannins from skins and seeds that attribute to appearance, taste and flavor of red wine. At the end of fermentation process, red wine is obtained when juice is flowed away by gravity, while pomace is collected from crushed grapes at this step. Most red wine, particularly in produce cool climate region, may be further treated to foster malolactic fermentation in order to reduce acidity [3]. This study focused on the red wine grape pomace (WGP) only since it contains more bioactive compounds than that of white wine grape pomace. Hence, all WGP refers to red WGP throughout the thesis.

2-2-2. Chemical composition in WGP

Pomace weights about 20% of the harvest grape [4]. WGP consists of approximately 30% seeds and 70% skins as well as minor parts of stems [5]. Compare to the stems, WGP seeds and skins have more oil, protein, pectin and sugar [6]. Therefore, only the grape seeds and skins are studied in this project. Although the chemical composition of WGP varies in the literature, those values were within comparable range on ash, fat, protein, soluble sugar, dietary fiber and polyphenol contents. Some studies pointed out that pectin and condensed tannin can be considered as part of dietary fiber, in which branched pectin represents as one-third of carbohydrate (uronic acid as rhamnose, arabinose and galactose) in soluble fiber fraction [6], whereas condensed tannin is related to the resisted protein in insoluble fiber fraction by the protein-binding capacity [7].

High ash in WGP skins is characterized as potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) [8], while phosphorus (P) is the major mineral in the seeds [9]. WGP seeds has abundant fat, mostly linoleic acid, followed by oleic, palmitic, stearic and myristic acids [7]. In respect to proteins, glutamic acid is the major amino acid along with limited lysine, tryptophan and sulfur-containing amino acids [10]. Glucose is the major soluble sugar in WGP [7] which varies by the extraction degree of winemaking [6]. Recent study found that WGP from French vineyard contains significant amount of glucans and xyloglucans, but lower pectinaceous polysaccharides, galacturonans and rhamnogalacturonans [11].

Dietary fiber and polyphenols are important bioactive compounds in WGP, and are the primary research interest in this study. Dietary fiber is the predominate fraction in dried WGP and its functionality will be discussed in Chapter 3-1. The ratio of insoluble to soluble dietary fiber fraction varies from 1.0 to 1.7 for fresh grapes [12], but WGP has significantly high values from 4.0 up to 22.5 [6-7, 10].

2-2-3. Phenolic compounds in WGP

The structures of the major phenolic compounds are presented in Figure 1. WGP contains either comparable or slightly higher total phenolic and flavonoid contents, but

lower amount of anthocyanins than that of fresh fruit extracts [13]. Overall, WGP has promising phenolic acids, including gallic acid and ellagic acid, and flavonoids, such as catechin, epicatechin, procyanidins and anthocyanins [14-15]. Lu and Foo (1999) detected 17 polyphenols in WGP by NMR spectroscopy [16], and Schieber, Kammerer, Claus and Carle (2004) were further identified 13 anthocyanins, 11 phenolic acids, 13 flavonoids, and 2 stilbenes in WGP by HPLC [17]. However, total phenolic content may be underestimated in some studies since most of the analytical methods are only targeting on soluble free phenolics, but exclude the bound phenolics, mainly in the form of β-glycosides [18].

WGP seeds generally exhibit higher polyphenol content than that in skins. It has been characterized as large quantities of monomeric phenolic compounds, such as (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-O-gallate, and dimeric, trimeric and tetrameric procyanidins [19]. On the other hand, WGP skins are rich sources of anthocyanins (mainly malvidin 3-*O*-glucoside, followed by peonidin 3-*O*-glucoside), hydroxycinnamic acids, and flavonol glycosides [17]. Rockenbach (2011) further quantified high concentration of flavonols (rutin and quercetin derivatives) in WGP skins from Brazilian winemaking [20]. Other phenolic compounds, such as chlorogenic acids (ester of caffeic acid and quinic acid), are presented in both WGP skin and seed extracts [20]. *Trans*-resveratrol (3,5,4'-trihydroxy-trans-stilbene) is below the detection level in WGP skins as it transferred from grape skin into red wine during fermentation, but is higher in WGP seeds due to the polar characteristic in seeds inhibits transfer activity [21-22].

2-2-4. Antioxidant activity of WGP

Phenolic compounds as secondary metabolites in plants attribute to both antioxidant and antimicrobial activities owing to their structure-activity relationships [15]. Yilmaz and Toledo (2003) reported that resveratrol is ranked as the highest peroxyl radical scavenging activity of phenolics in WGP, followed by catechin > epicatechin = gallocatechin > gallic acid = ellagic acid [23].

Figure 1. Structure of major phenolic compounds in WGP

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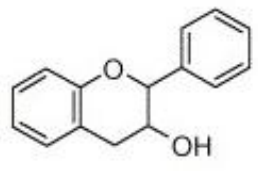
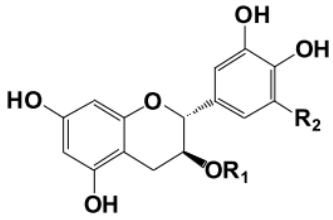
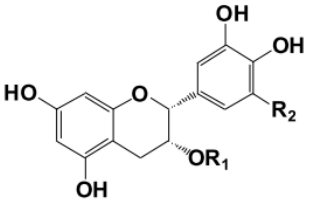
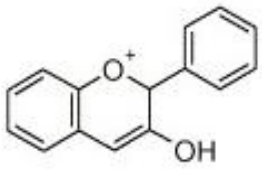
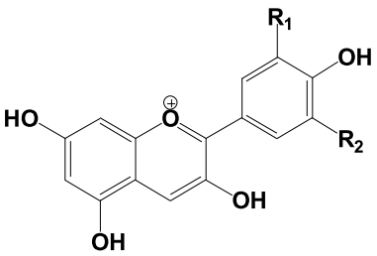
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td>a. Flavanol</td><td>(+)-Catechin: R1 = R2 = H</td><td>(-)-Epicatechin: R1= R2 = H</td></tr>
<tr><td>  </td><td colspan="2"> 
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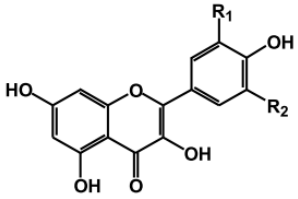
<tr><td>Delphinidin</td><td>-OH</td><td>-OH</td></tr>
<tr><td>Pelargonidin</td><td>-H</td><td>-H</td></tr>
<tr><td>Malvidin</td><td>$-OCH_3$</td><td>$-OCH_3$</td></tr>
<tr><td>Peonidin</td><td>$-OCH_3$</td><td>-H</td></tr>
<tr><td>Petunidin</td><td>-OH</td><td>$-OCH_3$</td></tr>
</table></td></tr>
<tr><td>b. Anthocyandin</td><td colspan="2">Major anthocyanins</td></tr>
<tr><td> 
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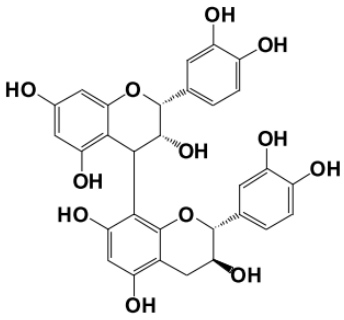
R2 = OH</td><td colspan="2"> 
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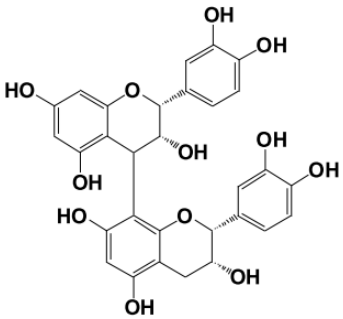
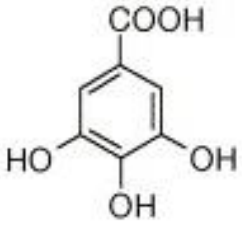
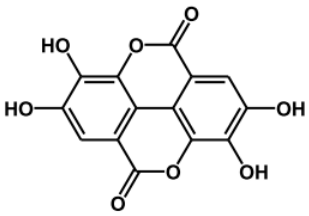
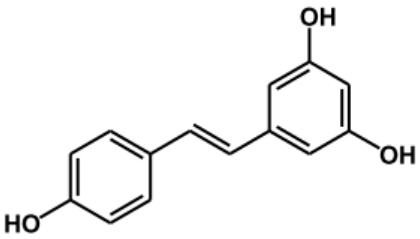
 
Prcyanidin B2</td></tr>
<tr><td>c. Flavonols</td><td colspan="2">d. Prcyanidin</td></tr>
</table>

<table>
<tr><td colspan="2">B. Phenolic acid</td><td>C. Other important polyphenols</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td>Gallic acid</td><td>Ellagic acid</td><td>Resveratrol</td></tr>
</table>

Source: Adapted from Balasundram and others [24] and Tsao, R. [25].

2-2-4.1. Polyphenol structure

Polyphenols based on the number of phenol rings and the structural elements bound to these rings have been classified into four categories, phenolic acids, flavonoids, stilbenes, and lignans. The first two groups is further introduced because they are the predominate polyphenols in WGP. Phenolic acids are classified into two subgroups: hydroxybenzoic acids with C6-C1 structure, such as gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid; and hydroxycinnamic acids containing aromatic compounds with three-carbon side chain (C6-C3), including caffeic acid, ferulic acid, *p*-coumaric acid and sinapic acid [26].

Flavonoids are the widest family of polyphenols. The backbone of low molecular weight flavonoid compounds are arranged in a C6-C3-C6 configuration, representing as two aromatic rings A and B, joined by a 3-carbon bridge to form a heterocyclic ring, C [24]. Flavonoid based on the substitution patterns to ring C is classified into flavonols, flavones, flavanones, flavanols (or named flavan-3-ol), isoflavones, flavanonols, and anthocyanidins [27]. Within each class of flavonoids, substitutions to rings A and B with oxygenation, alkylation, glycosylation, acylation, and sulfation also give rise to different compounds [27-28].

The antioxidant activities of phenolic compounds are contributed by their unique structure-activity relationships; that is, the numbers and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings [26]. In phenolic acids, gallic acid shows a high antioxidant activity due to trihydroxylated. On the other hand, flavonoid has more complicate structure-activity relationships. The catechol group containing ortho-dihydroxyl structure of ring B results in higher activity [29]. Catechin classified as group of flavan-3-ols or flavanols has catechol group on ring B, and epicatechin is the stereoisomer of catechin. Therefore, both (+)-catechin and (-)-epicatechin have strong hydroxyl [30], peroxyl [31], superoxide [32] and DPPH radical scavenging activities [33], and their radical scavenging activity is ten times higher than those of L-ascorbate and β-carotene [34]. In addition, Careri and others (2003) found that quertin as flavonol group also shows good antioxidant activity in WGP [21].

2-2-4.2. Antioxidant mechanism

Free radical is defined as an atom or molecule that posses an unpaired electron which could be anionic, cationic or neutral. Oxygen free radicals are the major free radical species because they play critical roles in the cell membrane destruction and food degradation. Oxygen free radicals belong to reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), lipid peroxide (LOOH), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl) and other N-Chloramine compounds. Others like carbonyl, thiyl and nitroxyl radicals are also important radical species. Phenolic compounds based on their structure-activity relationships present antioxidant activity under different mechanisms, such as free radical scavenging ability, hydrogen atom or electron donation, metal cation chelation, and singlet oxygen quenching [35].

Many *in vitro* methods have been used to evaluate WGP antioxidant activity, including measuring total phenolic compound by Folin-Ciocalteau (FC) assay, determining free radical scavenging activity by discoloration of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) assay or Trolox equivalent antioxidant capacity (TEAC) assay, testing the reducing power by ferric reducing antioxidant power (FRAP), and presenting the antioxidant capacity by oxygen radical absorbance capacity (ORAC) [36-38]. Among these analysis, FC assay for total phenolic content and DPPH assay for radical scavenging activity are most often used methods to investigate the antioxidant activity of WGP by considering polyphenols act as antioxidants that donate hydrogen to highly reactive radicals to prevent radical formation [39]. Total phenolic content analysis with FC reagent is easily oxidized and reacted to broader range of substrates targeting on both free and bound phenolics, while DPPH radical scavenging assay determines only free antioxidants in the extracts with various reaction speeds based on the sensitivity to specific compound [40-41]. Therefore, some phenolic antioxidants react to FC reagent may not express the reaction with the DPPH free radicals [41]. However, the free radical scavenging assay provides direct information on how capable an antioxidant can prevent reactive oxygen species from attacking lipoproteins, polyunsaturated fatty acids, DNAs, amino acids and sugars in biological and food systems [42].

2-2-5. Antimicrobial activity

The antimicrobial properties of polyphenols from WGP can be addressed by the degree of hydroxylation [43]. The hydroxyl groups on the phenolic compounds interact with the membrane protein of bacteria by hydrogen bonding that causes the changes in membrane permeability and cell destruction [44]. Özkan and others (2004) investigated that WGP extract can inhibit spoilage and pathogenic bacteria against *Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Escherichia coli O157:H7, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis, Salmonella typhimurium,* and *Staphylococcus aureus* [45]. Jayaprakasha and others [46] also reported that WGP has more efficient antibacterial activity on gram positive bacteria (inhibit by 850-100 ppm of WGP extract) than that of gram negative bacteria (1250-1500 ppm WGP extract).

In addition, resveratrol in WGP extracts are able to inhibit osmophilic yeast to prevent fungal food-borne contamination in apple or orange juices [47]. WGP as an antifungi agent can against *Z. rouxii and Z. bailii* due to the stilbenes content [42]. The research stated that although the stilbenes content is relatively low in WGP extracts, it is more active against the yeast than that of phenolic acids and flavonoids [42]. Furthermore, Thimothe and others (2007) indicated that WGP effectively inhibits virulence traits of *Streptococcus* mutans [13]. As a result, WGP extract as antibacterial, antifungal and antivirus activities is a good source of natural food preservative.

2-2. Preparation of WGP for further applications

WGP is mainly prepared in two ways for further applications: 1) dehydrate the fresh whole WGP and mill to obtain fine particles; 2) extract WGP and utilize in aqueous form. The amount of polyphenols in WGP are influenced by many factors, including the nature of WGP, grape cultivar, harvest time, growth climate and location [48], as well as the processing and storage conditions, extraction and analytical methods [15, 38, 49-50]. The objective of this study is to determine the retention and stability of phenolic compounds under different drying methods during long term storage under vacuum condition at 15°C.

2-2-1. Preparation of WGP for stabilization of phenolic compounds during storage

Fresh WGP after winemaking is perishable because high moisture content and high water activity cause the oxidation by increasing mobility of reactants and lead the loss of phenolic compounds [51]. Hatzidimitriou and others (2007) pointed out that under 75% of relative humidity, catechin and epicatechin contents in grape seeds reduced during 50 days of storage at 25 ℃ in the dark, but gallic acid was formed due to hydrolytic reactions [52]. Lavelli and Corti (2011) also indicated that the stability of phytochemicals in apple pomace degrades at water activity of 0.75 with following ranking: phloridzin > chlorogenic acid > quercetin 3-*O*-galactoside > epicatechin > procyanidin B2 and cyanidin 3-*O*-galactoside during 9 months of storage at 30 °C [53]. As a result, dehydration is the key step for preparation of WGP for further applications.

Bioactive compounds in WGP are heat and oxygen sensitive, and can be degraded by pH, polyphenol oxidase, sugar and organic acids [54]. Changes in polyphenol functions are irreversible after destruction, which are affected by energy transfer, oxygen availability, processing time and temperature, food composition and light exposure [55-56]. Thus, it is important to minimize the loss of bioactive compounds during food processing and storage. Although drying at lower temperature and pressure (such as vacuum and freeze drying) usually positively influences product qualities, they require longer processing time that raise the operation cost and energy. Therefore, the balance between bioactive compounds retention and cost/energy by different drying methods and condition is critical for industrial practice. Phenolic compounds of red WGP under different dehydration methods are shown in Table 2 and are briefly discussed in the following sections.

2-2-1.1 Mechanisms of thermal degradation of polyphenols

Dehydration is the process involving heat and mass transfer for moisture removal simultaneously. Degradation of nutrition and color during dehydration is demonstrated by the first order kinetic, $X = X_0 \exp(-kt)$ under constant process condition. At the beginning of drying, the mass transfer rate is generally high due to the evaporation of

surface moisture by external heat. The dehydration speed slows down afterwards when internal moisture is forced to transfer to the surface and evaporates until steady.

According to Maillard and Berset (1995), the reduction of phenolic content under thermal processing can be explained by three possible mechanisms [57]:

1) Partial degradation of lignins results in the release of phenolic acid derivatives;
2) Thermal degradation of the phenolic compounds;
3) Releasing of bound phenolic compounds. Because phenolic acids are mainly bound to carbohydrates and proteins, they could breakdown the cellular constituents and covalent bonds for release [58].

Nicoli, Anese, Parpinel, Franceschi, and Lerici (1997) reported that some new compounds are induced and formed under thermal treatment [59]. These compounds are the products from non-enzymatic browning or Maillard reaction, referred to as Maillard reaction products (MRPs), and can enhance antioxidant properties via a chain-breaking mechanism [59]. However, these MRPs are intermediate compounds and only act temporarily, so the acquired antioxidant activity from MRPs does not compensate for the loss of phenolic compounds [60-61].

2-2-1.2. Conventional oven dry

Drying at lower temperature for longer time is generally desirable because it reduces nutrient degradation on quality and retains the anthocyanin stability. Khanal and others (2010) reported that mild heating at 40 °C in conventional oven for 72 hours does not cause significant loss in anthocyanin, while using 125°C for 8 hours led 70% reduction [62]. High temperature causes adverse effect on color, flavor and nutrition value of food products [63]. Mildner-Szkudlarz, Bajerska, Zawirska-Wojtasiak and Górecka (2012) found that the stability of WGP phenols is as following: γ-resorcylic acid > gallic acid > tyrosol > catechin > isovanilic acid under baking temperature [64].

2-2-1.3. Vacuum oven dry

Vacuum dry produces higher polyphenols than that of hot air dry under similar temperature conditions because the low pressure reduces the drying time and minimizes

the oxidized bioactive compounds destruction [65]. Up to 95% of nutritious ingredients, vitamins and bioactive compounds from grape by-product preserved when subjected to vacuum dry at 500 mmHg and below 50 °C [66]. Vashisth and others (2011) investigated drying of muscadine pomace by vacuum belt at 22.50 to 60.00 mmHg, 60 °C for 60 minutes and no significant difference was observed in antioxidant activity compared to those by freeze drying, but reduced one-fourth of drying time [67]. The author also reported that vacuum belt dry can achieve the lowest water activity and moisture content compared to hot air dry and freeze dry [67].

2-2-1.4. Ambient air dry

The ambient air dry is usually conducted under ambient temperature in an open system along with a controlled speed of air velocity, so polyphenols are degraded because of polyphenol oxidase activity. Yilmaz and Toledo (2003) reported that total phenolic content is highly correlated with °Brix of the extracts when WGP was dried at 93 °C and 5 m/s air velocity within 90 min [23]. In addition, WGP drying at 60 °C and 2.3 m/s air velocity helped retain the polyphenolic content, color, and antioxidant activity of WGP skins. When subjected to temperature over 100 °C, extractable polyphenols are more sensitive than that of condensed tannin because condensed tannin has more complex chemical structure and is bound to fiber or protein [68].

2-2-1.5. Freeze dry

The principle of freeze dry is to freeze the liquid water into crystal phase, and then directly sublimate into vapor status to remove the moisture. Therefore, low temperature and low vacuum are the two critical conditions in freeze dry. The ice crystal formation in the plant tissue from freezing step results in cell wall puncture, which releases phenolic compounds into tissue matrix and easier to extract [69]. Since lyophilized WGP skin maintains the volatile, freeze dry is able to enhance the fruity aroma and color of poor harvest grape [70]. However, operating cost of freeze dry is high as it requires long dry time. Freeze drying yields the most polyphenols and reduces degradation and it has been

Table 1. Polyphenols and antioxidant activity of red WGP using different drying methods

Byproduct varieties/type	Drying methods	Drying Conditions	Polyphenols	Antioxidant activity	Biblo.
Cencidel/ Skin	Air-circulating oven	60°C, 8 h	EP 4.1%	FTC about 68%	68
		100°C, 3.5 h	EP 3.5%	FTC about 52%	
		140°C, 3 h	EP2.9%	FTC about 36%	
	Freeze dried		EP 4.3%	FTC about 72%	
Muscadine/ pomace	Vacuum belt dried	3-5 kPa, 60°C, 1 h	TPC 642 μmol GAE/g DW	FRAP 2.27 mmole Fe^{2+}/ g DM	67
	Hot air dried	70°C, 3 h	TPC 562 μmol GAE/g DW	FRAP 2.21 mmole Fe^{2+}/ g DM	
	Freeze dried		TPC608 μmol GAE/g DW	FRAP 2.30 mmole Fe^{2+}/ g DM	
Sunbelt/ pomace	Forced air oven dried	40°C, 72 h	ACY 1.076 mg/g DM		62
		60°C, 48 h	ACY about 1.000 mg/g DM		
		103°C, 16 h	ACY about 1.000 mg/g DM		
		125°C, 8 h	ACY about 0.950 mg/g DM		
	Freeze dried	14-16 h	ACY about 0.323 mg/g DM		
Merlot/seed	Air dried	93°C, 40 min	TPC 38.45 mg GAE/g DM	ORAC 344.8 μmol TE/g DM	14
Merlot/skin		93°C, 40 min	TPC 14.99 mg GAE/g DM	ORAC 69.8 μmol TE/g DM	
Chardonnay/seed		93°C, 60 min	TPC 32.13 mg GAE/g DM	ORAC 637.8 μmol TE/g DM	
Chardonnay/skin		93°C, 90 min	TPC 20.30 mg GAE/g DM	ORAC 102.8 μmol TE/g DM	

EP - extractable polyphenols; FTC- ferric thiocyanate method; TPC- total phenolic content; TFC- total flavonol content; ACY- anthocyanin content

considered as the reference to compare with other drying methods in some studies [68]. Although freeze dried samples retain the highest polyphenols compared to other drying methods, it still causes some losses [67, 71-72]. Therefore, some studies employed lyophilizing and powdering fresh WGP by liquid nitrogen directly to determine the maximum amount of phenolic compounds [20, 73].

2-2-2. Extraction of phenolic compounds

Solvent extraction involves diffusion process that uses liquid matrix (solvent) to liberate soluble phenolic compounds from solid matrix (grape tissue) [74]. Although many publications have brought up several solvent extraction methods for phenolic compounds from WGP, no agreement of extraction conditions has been reached. Solvent type, pH, extraction temperature and time and solvent-to-solid ratio are the major factors affecting the efficiency of solvent extraction. Methanol, ethanol, acetone, or ethylacetate are the most common organic solvents [75]. Ethylacetate extraction can obtain the higher phenolic purity, while ethanol can achieve higher yields for grape marcs [76]. In addition, acid hydrolysis improves degree of solubility. More phenolic compounds are released from the cell walls by adding acetic acid [62] or hydrochloric acid [73]. Extraction under higher temperature of 60 °C also enhances the phenolic yield, but apparent thermal degradation of constituents occurred after 20 hours extraction [77]. Previous studies applied the solvent-to-solid ratio from 1:1 to 10:1 along with the extraction time ranging from 30 minutes to 24 hours.

Recent studies have focused on using food grade solvents (water and ethanol) in combination with other novel methods to optimize extraction of phenolic compounds from grape byproducts [78]. Table 1 compares the polyphenol yields by using chemical solvents with other assisted extraction methods, including ultrasound [79-80] and microwave [81]. Ghafoor, Park, and Choi (2010) reported that the supercritical fluid extraction (SFE) is an efficient way to extract phenolic compounds [82], such as using supercritical CO_2 with ethanol as a modifier [83-84], or pressurized liquid extraction (PLE) from water [85]. Also, electrically assisted extraction has been studied, including high-voltage electrical discharges (HVED) [86], pulsed ohmic heating (POH) [87] and pulsed electric fields (PEF) [88].

Moreover, high hydrostatic pressure (HHP) significantly improves anthocyanins extraction from grape skins [89]. Corrales and others (2008) indicated that extraction from grape by-products assisted with ultrasound (35 KHz), high hydrostatic pressure (600 MPa) and pulsed electric fields (3 kV/cm) achieve 2, 3 and 4 fold higher antioxidant activities than that of extracted solely with solvent of ethanol:water 1:1 (v/v), liquid:solid 4.5:1 for 1 hour at 70 °C. Furthermore, other methods such as enzyme treatment, grindamyl pectinase and celluclast [90] and commercial pectinolytic [91], also enhance the extraction yield and recovery of phenolic compounds.

2-2-3. Stability of phenolic compounds during storage

Oxygen, pH, temperature moisture content, light, metal ions and enzymes are the main factors influencing the polyphenols storability [96]. No consistent trend has been shown in total flavonid content change during storage because the subclasses of flavonoid group revealed different stability. For instance, flavonol content was increased during cold storage for strawberries [97] and pears [98], but kaempferol was decreased for fresh strawberries after 3 months of storage in freezer [99]. Quercetin stability also has contradictory results in the previous researches that declines markedly in bilberries and lingonberries during 9 months of storage at 20 °C, but remained stable in black currants and red raspberries [99].

Anthocyanin stability is affected by temperature and light [100]. Anthocyanins increased during storage due to the synthesis of anthocyanin from the carbon skeletons by decrease in titratable acidity and organic acids [101]. Study has shown that anthocyanins in cranberries increased 3 to 5 fold compared to those of freshly harvested fruits during 3 months of storage at 15 °C [102]. Also, Kalt, Prange, and Lidster (1993) also reported that anthocyanin formation from strawberries during storage is greater at 20 °C than at 10 °C or 30 °C [103].

Table 2. Yield of phenolic compounds by different extraction methods for grape byproducts.

Extraction methods	Extraction conditions	Polyphenol amount	Biblo.
Traditional solvent extraction	50% methanol, then 70% acetone	TPC 26.3 mg GAE/g DM	[6]
	90% ethanol, 60 °C, 5 h	TPC yield rate 0.45% GAE	[76]
	Acetone: water: acetic acid (90:9.5:0.5)	TPC 462 mg CE/g DM	[92]
	Methanol: water: acetic acid (90:9.5:0.5)	TPC 381 mg CE/g DM	
Ultrasound assisted extraction (UAE)	Acetone: HCl: water (70: 0.1: 29.9), 3 h	TPC 26.7 mg GAE/g	[73]
	53.15% ethanol, 56.03 °C, 29.03 min	TPC 5.44 mg GAE/100 mL	[79]
Microwave assist extraction (MAE)	30 W. 66 °C, 200 sec	TPC 392 mg TAE/g extract	[81]
	500 W, 40% methanol, 100 °C, 5 min	ACY 1.9 mg/g	[93]
Supercritical fluid extraction (SFE)	Pressure 160-165 kg/cm^2 , 45-46 °C temperature, 6-7% ethanol as modifier	TPC 2.156 mg GAE/100 mL; ACY 1.176 mg/mL	[82]
Supercritical CO_2	350 bar, 8% ethanol, 35°C	TPC 52.6 ppm	[83]
Pressurized liquid extraction (PLE)	10 MPa, 0.1% HCl in water, 100 °C, 5 min	TPC 111.9 mg GAE/g DM; ACY 41.33 mg/g DM	[94]
	1400 μg/ml $Na_2S_2O_3$ in water, 110 °C, 40 sec	TPC 62.3 mg GAE/g DM	[85]
Pulsed ohmic heating (POH)	400 V/cm, 30 % ethanol, 50 °C, 60 min	TPC 8.9 mg GAE/g DM	[87]
High hydrostatic pressure (HHP)	600 MPa, 100% ethanol, 50 °C	ACY 32.8 mg/g DM	[89]
Enzyme assisted extraction	Grindamyl pectinase, 70% acetone, 8 h, enzyme/substrate (1/10)	TPC 605.5 mg GAE/100mL	[95]

TPC= total phenolic compound. ACY = anthocyanin content. GAE= gallic acid equivalent

2-2-4. WGP applications

High amount of WGP wastes generated from wineries cause economic and ecological problems. Many studies have been conducted in an attempt to convert this biowaste into environment-friendly applications [9, 96-97]. Traditionally, WGP has been used as animal feed [98] or as compost in Israel and Spain [99-100]. WGP was also applied as high-grade organic fertilizer for increasing the organic matter percentage, nutrient level, microbial biomass and improving the soil aeration and water-holding capacity in vineyards [96]. In addition, carboxyl, hydroxyl, sulphate, phosphate and amino groups from WGP proteins, carbohydrates and phenolics compounds can bind with metal ions to help remove toxic heavy metals from industrial wastewater, such as chromium, nickel and copper from aqueous solutions as low-cost absorbent [101].

WGP has been functioned as substrate for solid-state fermentation for ethanol [102], hydrolytic enzymes (cellullase, xylanase and exo-polygalacturonase) production [103], and pullulan extraction [104]. Moreover, WGP biocomposite boards were developed based on the thermoplastic properties of pectin, proteins, organic acids, and sugar in WGP [105]. Furthermore, WGP extracts based-edible films with was created as biodegradable packaging materials and showed antimicrobial and antioxidant functions for possible food applications [107]. For food applications, WGP has been further distilled and fermented to make traditional Mediterranean spirit, grappa [108]. WGP extracts have been applied for grape seed oil [109] and natural colorant [110-111]. Additionally, dietary supplements are launched based on high polyphenol in WGP seeds, extracts, and red wine powders in the US market [110]. Recently, WGP as functional food ingredient source of dietary fiber along with polyphenols have attracted great attention [112]. Food products fortified with WGP byproducts are further discussed in Chapter 3-3.

2-3. Red wine grape pomace as antioxidant dietary fiber

2-3-1. Dietary fiber

2-3-1.1. Definition, fractions and analysis of dietary fiber

Dietary fiber is the non-digestible plant cell wall material, primarily celluloses, hemicelluloses, pectin substances, gums, resistant starches (composed of four groups, RS1: physical inaccessible starch, RS2: ungelatinised starch granules, RS3: retrograded starch and RS4: chemically modified starch), other non-starch polysaccharides (e.g., polyphenols, waxes, saponins, cutin, phytates, resistant protein) and lignins [113]. According to the American Association of Cereal Chemists (AACC, 2001), dietary fiber is defined as "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 dietary fibers in cereals are primarily composed of celluloses, lignins and hemicelluloses, while in fruits and vegetables the principal sources are pectin, gum and mucilage [114]. The chemical nature of dietary fiber is complex due to the bond and degree of polymerization and the presence of oligosaccharide and polysaccharide residues [115]. Dietary fibers are classified as soluble (SDF) or insoluble (IDF) fractions based on whether they can be dissolved in water after enzymatic treatment or not. The former fraction includes pectin substances, gums, mucilages, and some hemicelluloses, whereas the later fraction is mostly celluloses, other types of hemicelluloses and lignins [116].

Qualification and quantification of the dietary fibers have been studied in the past decades. The non-enzymatic-gravimetric method was initially developed by Southgate and others (1978), but it underestimates the dietary fiber content without measuring the water soluble components [117]. The most common methods for dietary fiber analysis are the enzymatic-gravimetric method [118] and enzymatic-chemical method (including enzymatic-colorimetric and enzymatic-chromatographic methods) [119]. The key steps in the enzymatic-gravimetric method are to remove the starch and protein by enzymatic treatment, and then use ethanol precipitation to determine the filtration (soluble fraction) by liquid chromatography and residue weight (insoluble fraction) by correction for protein and ash [120]. Several AOAC Official Methods: 985.29 [121], 991.43 [122], 2001.03 [120] and 2002.02 [123] have been modified accordingly. The enzymatic-chemical method uses enzymes to remove starch and then to separate the soluble fraction by dialysis [124]. Both filtrate and residue are hydrolyzed to obtain the non-starch polysaccharide, in which the neutral sugars and uronic acids are determined by high-performance liquid

chromatography and colorimeter, respectively [119]. The residue, considered as Klason lignin, is the major part of insoluble fraction. However, this method may underestimate the dietary fiber amount due to the loss of polysaccharides during hydrolysis [125]. Since polyphenols related to polysaccharides and proteins in cell walls are considered as extension to other indigestible constituents, Goñi and others (2009) has updated the analysis for the dietary fiber associated polyphenols in foods and beverages [126].

2-3-1.2. Technological functionality of dietary fiber

Dietary fiber offers physiological functionalities on solubility, viscosity, hydration properties, oil-binding capacity and antioxidant activity in the food system. [113]. Insoluble fiber is characterized by porosity and low density [127], while the soluble fiber provides viscosity [128], gel forming ability and acts as emulsifier without loosen the texture and taste [113]. Solubility of dietary fiber is affected by temperature and pH and is increased with the presence of a substitution group of COOH and SO_4^{2-} [129]. Viscosity, the ratio of shear stress to shear rate, increases with increasing soluble fiber concentration but the decreasing of temperature [130]. Hydration properties (water holding capacity, swelling and water absorption on substrate pore volume) of dietary fiber are influenced by the polysaccharide's chemical structure, porosity, particle size, ionic form, pH, and temperature [113]. On the other hand, dietary fiber with high water holding capacity is able to avoid syneresis [131], in which fruit byproduct has less water affinity than that of algae [132]. Dietary fiber with high oil holding capacity is utilized for stabilizing high fat food product and emulsion. Zha and others (2009) pointed out dietary fiber is considered as antioxidant property that improves the oxidative stability and prolongs the shelf life of food products [133].

Therefore, fruit byproducts with rich in dietary fiber may be incorporated as partial replacement of flour, fat or sugar into food products as inexpensive and non-caloric bulking agents to improve emulsion or oxidative stabilities in food industry. However, the undesirable changes in color, texture and other properties should be concerned.

2-3-2. Antioxidant dietary fiber

2-3-2.1. Definition of Antioxidant Dietary Fiber

The concept of antioxidant dietary fiber (ADF) was first proposed by Saura-Calixto [134], and defined as a fruit or vegetable material that contains significant amounts of dietary fiber along with natural antioxidants. It should meet the following specific requirements:

a) Dietary fiber content should be higher than 50% on a dry matter basis measured by the AOAC method.
b) Free radical scavenging capacity at least 50 mg of vitamin E equivalent per gram of ADF measured by the DPPH method, or lipid oxidation inhibition capacity at least 200 mg of vitamin E equivalent per gram of ADF.
c) The above dietary fiber content and antioxidant capacity must be derived from natural constituents of the material.

Note that ADF is not a regulated term/claim by FDA or other agencies, but rather a term that has been used by researchers and industry for claiming its functionalities.

2-3-2.2. Different sources of ADF from Fruit Byproducts

Recently, dietary fiber from fruit and vegetable byproduct have been developed as food ingredients for improving texture, sensory characteristics and shelf-life on baked goods, beverages, confectionery, dairy, meat, pasta and soups [113]. The residual materials after fruit and vegetable processing remain rich dietary fibers content and antioxidant activity from their instinct properties. The main sources of fruit byproducts are grapes, apples, oranges, lemons, mangoes, peaches, apricots, pineapples, bananas and kiwifruits, while tvegetable waste is widely obtained from tomatoes, carrots, olive, red beets and potatoes [135]. In this literature, only fruit byproducts containing promising amount of both polyphenols and dietary fiber are discussed. The dietary fiber of each fraction along with total phenolic content and/or antioxidant activity from the fruit waste is summarized in Table 3.

Apple peels are about 25% of fresh apple weight and are one of the most important fruit byproducts. They have gelling and thickening properties due to the high amount of

soluble fiber that promotes pectin availability [136]. Apple peels are characterized for well balanced insoluble to soluble fraction of dietary fiber and high level of zinc, iron and copper that helps preventing atherosclerosis [137]. The major bioactive compounds in apple pomace include catechins, hydroxycinnamates, phloretin glycosides, quercetin glycosides, and procyanidins [138]. However, enzymatic browning is undesirable when adding apple peels into light colored food. Although bleaching apple pomace by alkaline peroxide treatment may prevent browning, it results in polyphenol loss and pectin degradation [139].

Orange is one of the most popular citrus fruits with unique color and taste, and it is a good source of carotenoids, flavonoids, essential oils, sugars, fibers and some minerals [140]. Orange peels and pulps are the waste after juice processing and weight about 50% of fruit mass. The insoluble dietary fiber in orange peel presents nutritional benefits to intestinal regulation and stool volume [141]. Fernández-López and others (2009) reported that flavanones (especially hesperidin), flavones (neodiosmin) and hydroxycinnamic (ferulic acid) are the major polyphenols in orange [152]. Lemon (*Citrus limon*) byproduct weights about 50% of original fruit after extracting juice and essential oil from its peels (albedo and flavedo), seeds and fruit pulps. Pectin extract from the lemon peels has been applied as gelling agent added into jams and jellies and as thickener, emulsifier and stabilizer in dairy products [153].

Mango (*Mangifera indica*) peels and kernels take part of 35% and 60% of total fruit weight. The mango peels are characterized of high amount of dietary fiber and extractable polyphenols [154], primary β-carotene [155], flavonol glycosides [156] and anthocyanin [157], while the mango kernels are mainly gallic acid, ellagic acid, gallate, gallotannin and condensed tannin-related polyphenols [158]. Ajila, Bhat and Prasada Rao [159] investigated that both ripen and unripe mango peels have good antioxidant activities because of the bioactive compounds, vitamin C and vitamin E content.

The kernels and peels from peaches (*Prunus persica*) contains pectins, dietary fibers and high amount of carotenoids, in particular β-carotene and β-crytoxanthin [160] but low level of α-carotene [161]. Apricot (*Prunus armeniaca L., Rosaceae*) pomace is a rich source of protein that has been applied as apricot seeds extracted oil for cosmetics and as dietary fat with no toxin detection for weanling rats [162].

Table 3. Examples of antioxidant dietary fiber from fruit byproducts

Byproduct	TDF	SDF	IDF	Polyphenol	Antioxidant activity	Biblio.
Grape pomace	61.32%	1.44%	59.88%	TPC 67.74 mg GAE/g DM	DPPH radical scavenge: 37.46 mg AAE/g	[142]
Grape peel	55.10%	1.40%	53.68%	TPC 32.35 mg GAE/g DM	DPPH radical scavenge: 33.43 mg AAE/g	[142]
Apple pomace	51%	14.60%	36.50%	TPC 10.16 mg/g		[143]
Apple skin	41%	31.30%	9.65%		FRAP assay: 7.62 mg TE/g DM	[144]
Orange peels	69%	50%	19%	TPC 1.6 mg/g DM	50% inhibition of oxidation of linoleic acid: 55.7	[145]
Lime peels	62%	40%	12%	TPC 3.5 mg/g DM	50% inhibition of oxidation of linoleic acid: 2.4 %	[145]
Mango peel	51.20%	19.00%	32.10%	TPC 96.2 mg GAE/g DM	DPPH Free radical scavenging IC 50: 79.6 mg	[146]
Guava peel	48.55%	1.83%	46.72%	TEP 77.9 mg GAE/g DM	DPPH EC50: 2.62 g/g DM	[147]
Guava pulp	49.42%	1.77%	47.65 %	TEP 26.2 mg GAE/g DM	DPPH EC50: 3.72 g/g DM	[147]
Pineapple shell	70.61%	0.51%	70.10 %	TPC 2.67 mg/g DM		[148]
Plums pomace	49.30%	36.20%	13.10%	TPC 6.86 mg/g DM	TEAC: 10.0 mikroM/g	[149]
Peach peel	32.67%	11.26%	21.93%	TPC 1.333 mg GAE/g extract		[131, 150]
Banana peel	83.00%	12.84%	70.16%	TPC 9.07 mg/g DM		[151]

TPC = total phenolic content, TPE= Total Extractable Phenol Content

Other fruit byproducts, such as pineapple pulp are used for ethanol production based on sucrose, starch and hemicellulose contents [163], whereas the pineapple shells provide good source dietary fiber and polyphenols [148]. Also, banana (*Musa×paradisiaca L., Musaceae*) peels take part of 30% of the ripe fruit with abundant source of anthocyanins (delphinidin, cyanidin, pelargonidin, peonidin, petunidin and malvidin) [164] and carotenoids (xanthophylls, laurate, palmitate or caprate) [165]. Moreover, guava (*Musa×paradisiaca L., Musaceae*) peels and pulps are considered as antioxidant dietary fiber but limited in pectin production [147]. Furthermore, kiwifruit pulps weights 30% of total kiwifruit crops containing about 25% of dietary fiber and the major phenolic compounds are characterized as phenolic acids, flavanol monomers, dimers and oligomers, and flavonol glycosides [166].

2-3-3. Benefits on WGP as antioxidant dietary fiber

2-3-3.1. Promotion of health benefit

Fruit byproducts attracted great interests for value-added application lately due to their promising amount of dietary fiber and polyphenols. Dietary fiber produce low molecular weight acids that are partially absorbed for energy [167]. The health benefits of dietary fibers includes reducing the risk of stroke, hypertension and coronary heart disease by lowering blood pressure and serum cholesterol levels, improving diabetes by regulating the blood glucose, benefiting certain gastrointestinal diseases (gastro-esophageal reflux disease, duodenal ulcer, diverticulitis, constipation, and hemorrhoids), promoting weight loss for obesity and appearing immune function enhancement from prebiotic fibers [168]. The general recommendation for adequate intake (AI) of dietary fiber is 14 g/1000 kcal/day [169]. Food and Nutrition Board from National Academy of Sciences recommends daily intakes (RDI) for dietary fibers are 21-26 and 30-38 g/day for adult women and men, respectively.

On the other hand, polyphenols refer as potential of antioxidant phytochemicals that are extranutritional constituents but limited quantities in foods [170]. Consuming phenolic compounds not only lower the risk of cardiovascular diseases and certain cancers by reducing low-density lipoprotein [171], but also have anti-tumor, anti-platelet, anti-allergic,

anti-ischemic, and anti-inflammatory functions in the human body [172]. In particular, gallic acid has benefits in retarding apoptosis [173], while flavonoid has anti-carcinogenic activity preventing colon cancer [174], breast cancer [175], ulcer [19] and anti-mutagenic activity [176]. However, flavonoids have been argued that inhibit iron absorption [26]. The antioxidant activity of WGP extract can suppress postprandial hyperglycemia for diabetic mice, specifically alpha-glucosidase inhibition [177], as well as prevention of oxidative stress and inflammation for induced-obese mice [178]. However, limited data showing the safe level or optimal amount of phenolic compounds intake for health [179]. Veskoukis and others (2012) indicated that the antioxidant effects from polyphenol-rich grape pomace extract do not correlated between *in vitro* (DPPH and ABTS radicals scavenging) and *in vivo* (oxidative stress using exercise as an oxidant stimulus) [180].

Epidemiological studies have pointed out that grape antioxidant dietary fiber (ADF) significantly increases the plasma antioxidant capacity [181]. Jiménez and others (2008) reported that the reduction in lipid profile and blood pressure from grape ADF is higher than those from other dietary fibers source (oat fiber or psyllium) due to the combined effect of dietary fiber and antioxidants [182]. Compared to fresh grape, pomace has higher contents of flavonoids, antioxidant vitamins A and E, and dietary fiber contents that promotes superoxide dismutase, catalase and glutathione peroxidase activities [183].

In earlier report, WGP as ADF was argued because did not change on hepatic antioxidant system against acetaminophen-induced oxidative stress was observed when feeding the rats [184]. The authors also noted that WGP evoked increase of the steady-state activity of glutathione peroxidase, but did not affect the activity of catalase, glutathione reductase and superoxide dismutase, nor the glutathione concentration in the liver[184]. However, other studies have presented that WGP as ADF not only retard human low-density lipoprotein oxidation *in vitro* [90], but also produce significant increase of beneficial *Lactobacillus* in rats cecum which may enhance the gastrointestinal health [185].

2-3-3.2. Prevention of lipid oxidation of foods

Lipid oxidation is one of the major concerns in food quality deterioration. The oxidative process may be catalyzed by light, heat, enzymes, metals, metalloproteins and

microorganisms, which may lead to off-flavor, loss of essential amino acids and fat-soluble vitamins. The most common lipid oxidation is autoxidation, meaning the unsaturated fatty acids generate free radical chain and proceed through three steps: initiation, propagation, and termination. Hydroperoxides (ROOH) are the primary products of autoxidation, while the secondary oxidation products are further decomposed from hydroperoxides, such as aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds. Peroxide value represented as total hydroperoxide content is an indicator of initial stage of oxidation, in which the iodometric titration assay is the one of the most common methods based on the oxidation of the iodide ion by hydroperoxides [187].

Antioxidants act various pathways preventing lipid oxidation, such as via binding metal ions, scavenging radicals, and decomposing peroxides. Since free radicals cause unsaturated lipid autoxidations in food, it is believed that phenolic hydroxyl groups can donate a hydrogen atom to those radicals and form stable end product in order to interfere with initiation or propagation for further lipid oxidation [188]. Synthetic antioxidants, such as BHT (buthylated hydroxytoluene), BHA (buthylated hydroxyanisole) and TBHQ (tert-butylhydroxyquinone), effectively retard lipid oxidation but raise concerns about safety and toxicity [189]. Therefore, WGP extracts may be applied as natural antioxidant to react with free radicals [46]. Previous study has shown that the antioxidant activity of WGP is comparable to BHT due to abundant polyphenolic substances [190].

WGP has demonstrated several benefits in food applications, including inhibition of toxic oxidation product formation, maintenance of nutritional quality, prevention of rancidity in lipid systems, and extension of food product shelf life. For example, WGP extract prevents the secondary oxidation products formation in sunflower oil [191] and the antioxidant effect is stronger than that of adding tocopherols in soybean oil [192]. Rababah and others (2011) developed WGP fortified corn chips that obtained lower peroxide value after storage [193]. In the seafood and meat industry, flavanol oligomers from WGP are the most potent oxidation inhibitors for emulsions in frozen fish muscles [194] and increased lipid stability in chicken breast [195-197].

2-3-4. Fruit byproduct as dietary fiber and antioxidant ingredients for food applications

2-3-4.1. Bakery products

Table 5 presents the dietary fiber and polyphenol contents and other functionality of the consumer products containing fruit byproducts. ADF is most commonly incorporated into bakery goods as the partial replacement of flour [198] to increase firmness and enhance the elasticity [199], without reducing in bread loaf volume [200]. Polyphenols contribute to the major antioxidant activity and improve product color, aroma and taste. Mildner-Szkudlarz, Zawirska-Wojtasiak, Szwengiel and Pacyński (2011) incorporated grape pomace into sourdough for rye bread making, and the final products received higher dietary fraction contents and radical-scavenging activities [201]. Also, grape seed flour applied to cereal bars, pancakes and noodles significantly improved the antioxidant activity of these products [202]. However, grape pomace reduced hardness and color deterioration when mixed into wheat biscuit [64].

Mango peel fortified biscuits improved the total dietary fiber and carotenoid contents. Water absorption increases as more amount of mango peel was powder added into the soft dough [146]. In addition, muffins incorporated with apple peels obtained higher water holding capacity [144]. Sudha and others (2009) also reported that the water absorption and extension values increase when apple pomace was fortified in cake [143]; however, apple pomace makes the dough become weaken because dough stability decreased and mixing tolerance index increased. Additionally, extruded orange pulp fortified biscuits help to increase water absorption and solubility index when replaced with the wheat flour [205].

2-3-4.2. Dairy products

Since dairy products are not considered as a significant source of polyphenols and dietary fiber, fruit is usually added to enhance the nutritional and functional properties. Dietary fiber as stabilizer helped ice cream bulk be more uniform and resist melting, as well as hinder of crystal growth when temperature fluctuates during storage [199]. Soukoulis and others (2009) found that dietary fiber from oat, wheat, apple and inulin

Table 4. Dietary fiber, polyphenol contents and other functional properties of food products fortified with fruit byproducts.

Byproducts	Food products	Functionalities	Biblo.
Red grape pomace	Rye bread incorporating 6% WGP- DF 15.05; TPC 4.80 mg/g	The hardness and gumminess of the bread increase whereas cohesiveness and resilience retain	[201]
White grape pomace	Wheat biscuits incorporation 10% WGP- DF 64.86%; TPC 2.11 mg GAE/g	Reduced water absorption and dough stability but not did affect dough development time	[64]
Mango peels	Biscuits incorporating 10% MPP- DF 14.4%; TPC 2.63 mg GAE/g	Increase in water absorption, carotenoid content and DPPH radical scavenage activity	[146]
Apple skin powder	Muffins incorporating 24% ASP- DF 7.6%; TPC about 0.75 mg GAE/g	Higher water holding capacity , less impact of thermal processing on antioxidant capacity	[144]
Apple pomace	Cake incorporating 25% AP- DF 14.2%; TPC 7.16 mg GAE/g	Increasing in water absorption and extension values, but cake volume decreases and dough gets weak	[143]
Orange pulp	Cookie incorporating 15% OP- DF 11.25%	The energy value decreased, but quality tends to be quite hard	
Grape seed	Cereal bars incorporating 5% GS- DPPF 10.73μmol TE/g d.m.	Grape seed flour from Merlot has better consumer acceptance than Cabernet Sauvignon	[202]
Grape pomace	Raw and chicken hamburgers incorporating 2% WGP	Lipid oxidation prevention is concentration-dependent under 13 days of refrigerated storage	[203]
Grape pomace	Minced horse mackerel muscle incorporating 4% WGP	Delay lipid oxidation during the first 3 months of frozen storage.	[204]

DF: dietary fiber, TPC: Total phenolic content

are able to control the crystallization and recrystallization in frozen dairy products [206].In yogurt, viscosity increases and the water absorption compensates are weak when high dose and large particle when orange fiber are added [207]. In addition, yogurt fortified with grape extract obtained good stability in the bioactive compounds and showed higher antioxidant power [208].

2-3-4.3. Other food products

Fernández-Ginés and others (2003) applied citrus fiber in bologna sausages that decrease in residual nitrite levels and extend the shelf-life by delaying lipid oxidation [209]. Also, grape pomace as antioxidant dietary fiber added in raw and chicken hamburgers improves the oxidative stability and the radical scavenging activities [203]. In addition, cereal or fruit (peach, apple and orange) dietary fiber as fat substitute in fermented sausages not only reduces the caloric content and improves the texture and stability, but also reveals similar characteristics on sensory evaluation [210]. In seafood products fortified with fruit or chitosan, soluble fibers improve water binding, thickening, emulsion capacity and gelling properties , but lose rigidity and elasticity in muscle protein gels [211]. Moreover, grape pomace as antioxidant dietary fiber plays an important role in delaying the oxidation and improving flavor in the minced fish [212] and restructured fish [204].

2-4. Conclusion

Wine grape pomace (WGP) with promising amount of polyphenol and dietary fiber content may be employed as functional food ingredient that not only delays lipid oxidation and extends the shelf-life of food products, but also promotes human health by lowering the risk of several diseases. The concept of antioxidant dietary fiber (ADF) has been launched in research and industrial purpose for claiming the beneficial functionalities. Thus, it is worth to investigate if WGP can meet the definition of ADF.

WGP is usually dehydrated and stored under vacuum or low moisture conditions before further applications. Limited information on how different drying methods affect the bioactive compounds retention in WGP during long term storage stability was studied.

Therefore, it is important to investigate the economic feasible drying method that can help retaining the polyphenols under storage since those compounds contribute to both antioxidant and antimicrobial activities.

Fruit byproducts may be utilized as functional food ingredient as a safe, useful, natural, health promoting antioxidants. Meanwhile, some important food characteristics may change due to the functional properties on dietary fiber. As a result, another objective in this project was to develop WGP fortified in high value dairy product (yogurt) and high oil emulsified food system (salad dressing) with the well balanced dietary fiber and phenolic content, as well as physicochemical and sensory qualities for the products.

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Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot)

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ABSTRACT

The effects of different drying methods (40 °C conventional and vacuum oven, 25 °C ambient air and freeze dry) on the stability of two red wine grape (Pinot Noir, PN and Merlot, M) byproducts, pomace containing skins and seeds (P) and pomace containing skins only (S) were investigated. Freeze dried samples retained the highest bioactive compounds with total phenolic content (TPC) of 21.19-67.74 mg GAE/g d.m., anthocyanin content (ACY) of 0.35-0.76 mg Mal-3-glu/g d.m., DPPH antiradical scavenge activity (ARS) of 22.01-37.46 mg AAE/g d.m., and total flavanol content (TFC) of 30.16-106.61 mg CE/g d.m., followed with ambient air dried samples. All samples lost significant amount of bioactive compounds during 16 weeks of storage at 15±2 ^{o}C, in which ambient air and freeze dried samples had TPC reduction of 32-56% and 35-58%, respectively, but ARS in PN-P and M-P still remained more than 50 mg TE/g d.m. Overall, TPC, ARS and TFC were higher in PN than in M, and higher in pomace than in skins, while reverse results were observed in ACY. Pomace extracts showed higher antibacterial efficiency against *L. innocua* than *E. coli* with minimal inhibition concentration (MIC) of 3, 6, 4 and 9% against *E. coli*, and 2, 7, 3 and 8% against *L. innocua* for PN-P, PN-S, M-P and M-S samples, respectively. Dietary fiber content of samples was 57-63% of total dry matter. This study demonstrated that Pinot Noir and Merlot pomace are good sources of antioxidant dietary fibers and may be incorporated into various food products as a functional ingredient.

Key words: wine grape pomace, drying methods, antioxidant dietary fiber, stability, antimicrobial activity

Practical application

Wine grape pomace, the byproduct of wine making, is a good source of polyphenols and dietary fibers and may be incorporated into various food products as a functional ingredient. This study reported the effect of four drying methods and storage at 15±2 ^{o}C up to 4 months on the retention of polyphenols and antioxidant activity in two types of red wine grape pomace (with and without seeds). Antibacterial activity, dietary fiber content and the basic physicochemical properties of dried pomace powder were also reported. The information is essential for developing specific applications of the pomace.

Introduction

Wine grape pomace (WGP), the byproduct from wine processing, weighs about 20% of the harvest grapes (Laufenberg and others 2003). There are increased interests in converting this cheap biowaste into value-added products, such as extraction of bioactive compounds as dietary supplements and grape seeds oil for promoting human health (González-Paramás and others 2003; Maier and others 2009). Our previous studies investigated the feasibility of creating edible films using WGP extracts by utilizing the residual pectin, cellulose and sugars (Deng and Zhao 2011), and developed WGP biocomposite boards based on the thermoplastic properties of pectin, proteins, organic acids, and sugar in WGP (Park and others 2010). In addition, the chemical composition of polyphenols and dietary fiber in red and white WGP from the U.S. Pacific Northwest were characterized (Deng and others 2011).

WGP are rich source of phenolic acids that in wine grape seeds contain a great amount of monomeric phenolic compounds (Guendez and others 2005). Those compounds contribute to both antioxidant and antimicrobial activities, and have been shown to act as the free radicals scavenge to inhibit low-density lipoprotein oxidation and certain types of cancer (Yildirim and others 2005). Dietary fiber is another predominate functional component in WGP, and has benefits of reducing the risk of cardiovascular diseases, cancers, and diabetes (Lizarraga and others 2011).

WGP are good sources of both polyphenols and dietary fibers, thus has been claimed as antioxidant dietary fiber (ADF), a concept first proposed by Saura-Calixto (1998) and further reported by several other studies (Llobera and Cañellas 2007). In brief, ADF is defined as a product containing significant amount of natural antioxidants associated with the fiber matrix. Specifically, the dietary fiber content of any ADF should be higher than 50% dry matter, and 1 g of ADF should have capacity to inhibit lipid oxidation and DPPH free radical scavenging capacity equivalent to vitamin E at least 200 mg and 50 mg, respectively (Saura-Calixto 1998).

Dehydration of wet pomace is a first step before developing further applications. However, polyphenolics are sensitive to heat and oxygen. Several studies have evaluated the effects of different drying methods on the biochemical changes of fruit pomace (Khanal and others 2010; Vashisth and others 2011). The minimum loss of bioactive

compounds were found at drying temperature not higher than 50 °C (Raghavan and Orsat 2007). For fully benefit from WGP, it is critical to develop drying conditions that can maximize the retention of polyphenolics while remaining economically feasible.

The aims of this study were to investigate economically feasible drying methods, including 40 °C conventional oven, 40 °C vacuum oven, and 25 °C ambient air in comparison with the most effective but expensive freeze-dry, and to evaluate the stability of the bioactive compounds in dried WGP during 16 weeks of storage at 15±2 ^{o}C. Red WGP from two predominate red wine grapes in US northwest pacific area, Pinot Noir and Merlot, were evaluated. Pomaces containing both seeds and skins and with only skins were investigated. Additionally, the antibacterial activity of pomace extract against Gram-positive and Gram-negative bacteria was determined based on the minimum inhibition concentration. Moreover, the physiochemical properties and chemical composition of dried pomace were analyzed.

Materials and Methods

Materials

Two varieties of red wine grape pomace, *Vitis vinifera* L. cv. Pinot Noir and cv. Merlot were acquired from Oregon State University Research Winery (Corvallis, OR, USA). Stems were manually removed from pomace to collect seeds and skins, after which the seeds were further separated manually to obtain only skins. In this study, pomace containing skins and seeds named as pomace (P) while pomace containing skins only names as skin (S). Therefore, four different red WGP samples were evaluated, including Pinot Noir seeds and skins (PN-P), Pinot Noir skins only (PN-S), Merlot seeds and skins (M-P), and Merlot skins only (M-S). All samples were subjected to four different drying conditions: 40 °C forced-air oven (Thermo Fisher Scientific Inc, USA), 40 °C vacuum oven (Forma Scientific Inc., USA) with vacuum of 27 Pa, 25 °C air dry at room temperature, and freeze dry at -55°C and vacuum of 17.33 Pa (Model 651 m-9WDF20, Hull Corp., Hatboro, USA) until no further weight loss. It took about 48 h to dry about 500 g of fresh pomace or skins using 40 °C forced-air oven or vacuum oven, but 72 h and 60 h when drying at room temperature and freeze dry, respectively. Dried sample was ground (Gien Mills Inc., USA) with particle size of 0.85 mm. The powders

were then vacuum-packaged (Food Saver Vac 1075, Tilia Inc., USA) and stored at controlled temperature of 15 ± 2°C to analyze the bioactive compounds, and antibacterial activity at week 0, 8 and 16 under dark.

Physicochemical properties of dried pomace powders

Moisture content of dried powders was determined by drying samples in 105 °C oven (STM 40, Precision Scientific Inc., USA) until reaching consistent weight, and the percentage of weight loss was calculated as wet based. Water activity was measured using the AquaLab water activity meter (Decagon Device, Inc., USA). Color was monitored using a colorimeter (Lab Scan II, Hunter Associate Laberatory Inc., USA), in which the white color plate with X: 78.25, Y: 82.85, Z: 85.83 and 10° standard observer was used for calibration. Samples were placed inside a glass refract cup on the light pore size of 44.45 mm. Data were recorded as L*, a*, b* values.

Analysis of bioactive compounds of dried pomace

Sample extraction

Pomace powders were extracted by 70% acetone /0.1% HCl /29.9% water (v/v/v) at a solvent to pomace powder ratio of 4:1 (v/w) (Deng and others). The mixture was placed in ultrasonic unit (Branson B-220H, SmithKline Co., USA) for 60 min, and centrifuged (International Equipment Co., USA) at 10,000 g for 15 min. This procedure was repeated for three times. All supernatants were then combined and concentrated using a rotation evaporator (Brinkmann Instruments, USA) at 40 °C. The extraction yield for the pinot noir pomace was about 8% after freeze dried the extract. It was anticipated that all samples had similar extraction yield. The final extracts were stored in a -70 $^{\circ}$C freezer until analysis.

Analysis of total phenolic, anthocyanin, antiradical scavenging activity, and flavanol content

Total phenolic content (TPC) was determined by the Folin-Ciocalteu assay (Singleton and Rossi 1965). The diluted extract was reacted with Folin-Ciocalteu reagent (Sigma Chemical Co., MO, USA) for 10 min, and then incubated with 20% $NaCO_3$ in 40 °C water bath for 15 min. Absorbance was measured spectrometrically at 765 nm (UV160U,

Shirmadzu, Japan). Gallic acid (Sigma Chemical Co., USA) was used as a standard, and results were expressed as mg gallic acid equivalents (GAE)/g extract.

Anthocyanin content (ACY) was measured by the pH differential method (Giusti and Wrolstad 2001). The extract was both diluted with pH 1.0, 0.025 M potassium chloride and pH 4.5, 0.4 M sodium acetate. The mixtures were measured spectrometrically at both 520 nm and 700 nm. Thus, anthocyanin content (mg Mvd-3-glu equivalents/g extract) was calculated as $\frac{((A520,pH\ 1-A700,pH\ 1)-(A520,pH\ 4.5-A700,pH\ 4.5))\times 529\times \text{dilute factor}\times 1000}{28000}$, where the molar absorptivity and molar mass of malvidin-3-glucoside (Mvd-3-glu) were 28,000 L/cm/mol and 529 g/mol, respectively. Results were expressed as mg Mvd-3-glu equivalents/g extract (Thimothe and others 2007)

Antiradical scavenging activity (ARS) was determined by 1,1-diphenyl-2-picryhydrazyl (DPPH) (Kasel Kogyo Co. Ltd, Japan) assay (Brand-Williams and others 1995). The diluted extract was reacted with DPPH-methanol reagent (9 mg DPPH in 100 mL methanol) for 10 min at room temperature. Ascorbic acid (Mallinckrodt Baker Inc., USA) was applied as a standard and the results were expressed as mg ascorbic acid equivalents (AAE)/g extract at absorbance of 517 nm. In addition, α-tocopherol standard curve was determined and expressed as mg α-tocopherol (TE)/g extract.

Total flavanol content was measured by vanillin (Alfa Aesar, USA) assay along with spectrometer (Price and others 1978). Briefly, extract and (+)-catechin hydrate (Sigma Chemical Co., USA) standard were mixed with two solutions. One set was mixed with of 4% HCl-methanol (v/v). Another set was mixed with vanillin reagent (0.5% vanillin in 4% HCl-methanol, w/v) at 30 °C water bath for 20 min. The different absorbencies between the two solutions were measured spectrometrically at 500 nm and expressed as mg catechin equivalents (CE)/g extract.

Antibacterial activity of pomace extracts

Freeze dried powders were further evaluated for their antibacterial activity and chemical compositions. Minimum inhibition concentration (MIC) was investigated as indicator of antibacterial activity. Same pomace or skin extract used for the bioactive compound analysis was used for the study. *Escherichia coli* ATCC 25922 and *Listeria innocua* ATCC 51142 were applied to evaluate the antibacterial efficiency against gram-

negative and gram-positive bacteria. *E. coli* and *L. innocua* were enriched in Tryptic Soy broth (TSB) and Brain Heart Infusion (BHI) broth (EMD bioscience, USA), respectively, overnight at 37 °C to reach 10^7 cfu/mL. Extracts were added into the BHI and TSB to make the concentration ranged from 1% to 15% (0% was considered as the control) in sterile test tubes with 20 μL of bacteria enrichment. After being placed in the incubator at 37 °C overnight, samples were diluted serially with phosphate-buffered saline (EMD bioscience, USA) and enumerated by placing in TSB agar for 24 h (for *E. coli*) and BHI agar for 48 h (for *L. innocua*) at 37 °C to count the colony forming unit. The results were expressed as the least percentage of extract concentration that achieved the significant reduction (based on LSD at 95% confidence level) in population of *E. coli* or *L. innocua* compared to control.

Chemical composition of dried pomace

Ash, protein, and fat content

Ash content was determined at 525 °C muffle furnace for 5 h (AOAC 942.05). Protein was analyzed by the micro-Kjeldahl method and calculated as nitrogen factor of 6.25 (AOAC 960.52). Crude fat was extracted with petroleum ether by the Soxhlet system under 60 °C for 6 h (Murthy and others 2002).

Soluble sugar

After the powders were extracted by 80% ethanol for 15 min and centrifuged at 10,000 g for 10 min three times, the supernatants were collected and concentrated by rotation evaporator at 50 °C. Soluble sugar was determined by the anthrone method of using D-glucose (Sigma Chemical Co., USA) as standard (Goñi and others 2009). Samples were mixed with 75% sulfuric acid and anthrone (Alfa Aesar, USA) reagent at 100 °C for 15 min, and read the absorbance at 578 nm.

Dietary fiber

Dietary fiber of the powders we analyzed by the enzymatic-gravimetric method (AOAC 994.13) with modifications (Deng and others 2011). Briefly, samples were treated with protease (P-5459, Sigma Chemical Co., USA) in 0.05 M, pH 7.5 phosphate buffer at 60 °C for 30 min. Soluble dietary fiber (SDF) was obtained from the supernatant after centrifuge, while insoluble dietary fiber (IDF) was the residues.

Dialysis for SDF used the tubing with a molecule weight cutoff of 12,000-14,000 (Spectrum Laboratories, Inc., USA) in deionized water for 48 h. The dialysate was freeze dried and hydrolyzed with 72% sulfuric acid at 121^{o}C for 1 h. Neutral sugar (NS) determination was based on the anthrone method as description in soluble sugar above. Uronic acid (UA) was quantified as galacturonic acid (Spectrum Chemical, Co., USA) equivalent along with spectrometric assay. The mixture of sample, 98% H_2SO_4 and boric acid-sodium chloride solution was incubated at 70 °C for 40 min. Solvent was then treated with 3,5-dimethyphenol-glacial acetic acid (Sigma Chemical Co., USA), the absorbance was read at 400 and 450 nm, respectively.

IDF residue was hydrolyzed by 72% sulfuric acid at 30 °C for 1 h with stirring, and then at 121 °C for 1 h. The mixture was filtrated by fritted crucible (Corning, Inc., USA). The amount of IDF was quantified by the sum of NS and UA from the solution after filtration as described above, and included Klason lignin (KL) and ash which were residue remaining in the crucible. KL was determined by the mass change after drying for 16 h at 105 °C. Ash and resistant protein (RP) were measured using the same procedure as ash and protein analysis described above. Total dietary fiber (TDF) was calculated as the sum of SDF and IDF.

Condensed tannin

Dried powders were treated with protease in 0.05 M, pH 7.5 phosphate buffer as described for the analysis of dietary fiber (Reed and others 1982). The residue was incubated in 5% HCl-butanol (v/v) at 100 °C for 3 h and at 533 nm absorbance was measured. The condensed tannin prepared from wine grape skin (~80.4% by weight CT) was used as a standard.

Pectin

Pectin was quantified by the sum of water soluble, chelator soluble and hydroxide soluble pectin as described by Silacci (1990). Briefly, pomace was mixed with water and homogenized for 10 min and the slurry was filtered in a Buchner funnel. The 95% ethanol was then added into the filtrate to precipitate overnight at 40 $^{\circ}$C for obtaining the water soluble fraction. The residue was washed with 20 mM, pH 8.0 disodium ethylenedinitrilo tetraacetic acid (Mallinckrodt Baker, Inc., USA) and boiled sequentially for three times, and all filtrate was then collected for measuring chelator soluble fraction.

The residues were further treated with 50 mM NaOH to obtain the hydroxide soluble pectin fraction. Each pectin fraction was analyzed by the UA protocol as described in the determination of SDF.

Experimental design and statistical analysis

Physiochemical properties and bioactive compounds of dried pomace samples were determined with four replications. Analysis of variance (ANOVA) was performed to evaluate significant treatment effect of three independent factors including drying methods, wine grape varieties and the types of byproduct, as well as their possible interactions. Antimicrobial activity and chemical compositions of the samples were tested with triplications and the mean values were compared based on LSD at 95% confidence level. All data were analyzed by general linear model procedure (PROC GLM) of SAS 9.2 (SAS Inst. Inc., USA).

Result and Discussion

Physicochemical properties

Water activity (Aw) of dried samples ranged from 0.14 to 0.42 (Table 1). Overall, air dry had the highest Aw of 0.28 to 0.42, while vacuum dry had the lowest Aw of 0.14 to 0.21. In general, there was no significant ($P>0.05$) difference between Piont Noir and Merlot, except lower Aw values in vacuum dried PN-P, air dried M-S and freeze dried M-P. Also, there was no significant ($P>0.05$) difference in Aw between pomace and skin samples in two wine grape varieties, except Aw of air dried M-P and freeze dried PN-P were higher than their skin samples. These results were consistent with previously reported Aw of 0.20-0.23 in wine grape pomace (Monagas and others 2005). Lavelli (2011) indicated that apple pomace at high water activity level of 0.75 lost all its phytochemicals during storage. Therefore in this study, all dried pomace samples were vacuum packaged in moisture barrier bags for preventing the water absorption during storage.

Moisture content (MC) of all dried samples was between 4.40 to 7.65% (Table 1), in which air dry retained the highest MC. Drying method did not significantly ($P>0.05$) affect MC in skin samples, but air drying resulted in the highest MC in pomace. Overall,

there was no significant difference between Pinot Noir and Merlot, except vacuum dried PN-P and freeze dried M-S showed lower MC. Moreover, MC was higher in vacuum and air dried M-P, and freeze dried PN-P and M-P compared with other dried skin in both varieties. These results were consistent with previously reported MC of 5.1% and 5.4% in grape skins dehydrated at oven 60 °C and freeze dried, respectively (de Torres and others 2010).

Freeze dry received the highest L* value of 32.80-46.64 compared to other dried samples, which ranged 27.22-40.21. Among different varieties, L* values were higher (lighter color) in Pinot Noir than in Merlot, except air and freeze dried PN-S. Also, skin samples obtained lighter color (higher L* value) than pomace in both varieties. These values were consistent with previously reported result that freeze dried red grape pomace peels had higher L* value of 46.9 than 31.0 of oven dried sample at 60 °C (Larrauri and others 1997).

The a* values followed the same tendency as L* with the highest value 10.94-11.65 in freeze dried ones, while samples dried by other methods ranged 7.53-10.21. In comparison with Pinot Noir, Merlot had higher a* values in both pomace and skin. Visual observation also showed that Pinot Noir was darker, and Merlot was more toward red. Moreover, pomace obtained higher a* values than skins in both varieties. For the b* values, freeze dried Pinot Noir had the highest value, Pinot Noir showed higher b* values than that of Merlot, and pomace had higher b* values than skins among different drying methods in both varieties ($P<0.05$). PN-P had high b* values 7.87-10.20, while M-S had low values of 1.29-1.66. Liang (2011) investigated the relationship between CIELAB parameters and anthocyanin content in different varieties of berry skins, and indicated that a* values are positively correlated with anthocyanins, but L* and b* values are negatively correlated.

Effect of different drying methods on the bioactive compounds and antioxidant activity

As shown in the ANOVA table (Table 2), the contents of total phenolic (TPC), anthocyanin (ACY), antiradicals scavenge activity (ARS) and total flavonol (TFC) were significantly affected by drying methods, wine grape varieties and types of byproduct

Table 1. Physiochemical properties of Pinot Noir and Merlot pomace and skin samples dried by different methods

Parameters	Drying methods	Pinot Noir		Merlot	
		Pomace	Skin	Pomace	Skin
Aw	Oven at 40 °C	BC 0.19 ± 0.02 a	B 0.23 ±0.04 a	B 0.18 ± 0.24 a	BC 0.22 ± 0.04 a
	Vacuum at 40 °C	C 0.14 ± 0.01 b	C 0.17 ± 0.02 b	B 0.21 ± 0.00 a	C 0.19 ± 0.02 ab
	Air at 25 °C	A 0.42 ± 0.13 a	A 0.38 ± 0.01 a	A 0.38 ± 0.07 a	A 0.28 ± 0.02 b
	Freeze dry	B 0.29 ± 0.06 a	B 0.25 ± 0.02 b	B 0.23 ± 0.03 b	AB 0.25 ± 0.04 b
Moisture Content	Oven at 40°C	B 5.94 ± 0.08 a	A 5.92 ± 1.07 a	B 5.77 ± 0.19 a	A 5.24 ± 0.94 a
	Vacuum at 40°C	C 5.24 ± 0.05 b	A 4.99 ± 0.36 b	B 6.26 ± 0.21 a	A 4.93 ± 0.27 b
	Air at 25 °C	A 6.71 ± 0.03 ab	A 6.95 ± 1.34 ab	A 7.65 ± 0.44 a	A 5.10 ± 0.35 b
	Freeze dry	B 6.09 ± 0.09 a	A 5.60 ± 0.22 b	B 6.10 ± 0.09 a	A 4.40 ± 0.05 c
L*	Oven at 40 °C	C 29.63 ± 0.37 b	C 36.32 ± 0.41 a	B 29.57 ± 0.64 b	B 37.52 ± 0.71 a
	Vacuum at 40 °C	B 33.53 ± 0.75 c	B 40.21 ± 0.08 a	B 29.80 ± 0.02 d	B 37.94 ± 1.15 b
	Air at 25 °C	B 32.96 ± 0.13 b	B 39.42 ± 0.64 a	C 27.22 ± 1.10 c	AB 39.80 ± 1.01 a
	Freeze dry	A 43.32 ± 0.35 b	A 46.64 ± 0.57 a	A 32.80 ± 0.20 d	A 41.66 ± 0.69 c
a*	Oven at 40 °C	B 8.30 ± 0.16 c	C 7.53 ± 0.26 d	B 10.17 ± 0.17 a	B 9.41 ± 0.14 b
	Vacuum at 40 °C	B 9.08 ± 0.88 b	BC 8.17 ± 0.25 b	A 10.21 ± 0.02 a	B 8.96 ± 0.31 b
	Air at 25°C	B 8.89 ± 0.12 ab	B 8.42 ± 0.06 bc	C 9.58 ± 0.03 a	B 9.24 ± 0.30 a
	Freeze dry	A 11.34 ± 0.12 ab	A 10.94 ± 0.34 b	A 12.49 ± 0.04 b	A 11.65 ± 0.04 a
b*	Oven at 40 °C	B 7.87 ± 0.19 a	B 5.13 ± 0.11 b	A 6.91 ± 1.44 ab	A 1.66 ± 0.11 c
	Vacuum at 40 °C	A 10.10 ± 1.36 a	B 5.36 ± 0.29 b	A 6.85 ± 0.13 b	B 1.29 ± 0.02 c
	Air at 25 °C	AB 9.03 ± 0.05 a	B 5.50 ± 0.10 b	AB 9.47 ± 0.13 c	AB 1.38 ± 0.18 d
	Freeze dry	A 10.20 ± 0.21 a	A 7.08 ± 0.27 b	B 5.87 ± 0.06 c	AB 1.56 ± 0.02 d

Means followed by the same capital letters (A – D) in the same column within each type of drying methods were not significantly different ($P > 0.05$). Means followed by the same lowercase letters (a – d) in the same row within each varieties and byproducts were not significantly different ($P > 0.05$).

($P<0.05$). There were interaction effects between drying method and type of byproduct on TPC and TFC, and between wine grape variety and type of byproduct on TPC, ACY and TFC. Lu and Foo (1999) had studied the individual polyphenol constituents of grape pomace by HPLC and Rubilar and others (2007) further found that WGP contained phenolic acids, phenolic alcohol, flavan-3-ols and flavonoids. Since gallic acid, monomers catechin and epicatechin are the main phenolic compounds in grape seeds and anthocyanins (mainly malvidin-3-glucoside) was found in grape skins, gallic acid equivalent, catechin equivalent and malvidin-3-glucoside equivalent were used to represent total phenolic content, total flavanol content and anthocyanin content, respectively in this study, to investigate their changes by different drying methods and during storage.

Freeze dry retained the highest amount TPC in all samples compared to other drying methods (Fig. 1). Immediately after drying, TPC of freeze dried PN-P and M-P were 67.74 and 40.98 mg GAE/g d.m., while PN-S and M-S were 32.35 and 21.19 mg GAE/g d.m., respectively. There was no significant ($P>0.05$) difference in TPC among other three drying methods with the value ranging 41.07-44.74 and 23.88-30.40 mg GAE/g d.m. for PN-P and M-P, and 20.87-23.58 and 15.72-18.08 mg GAE/g d.m. for PN-S and M-S, respectively. Also, Pinot Noir contained higher TPC than Merlot as it has been well known that different cultivars, harvest time, location and growth environment affect TPC of wine grapes (Lee and others 2005). Moreover, TPC in pomace was significantly ($P<0.05$) higher than skins in both varieties. Makris (2007) reported that TPC of red grape pomace and skin were about 23.99 and 15.02 mg GAE/g d.m., respectively. Seeds were identified to contain abundant phenolics, such as gallic acid, catechin, and epicatechin (Alonso and others 2002). Koo (2007) also confirmed that TPC in wine grape pomace after winemaking fermentation is slightly higher than whole fruit extract, with TPC of 61.8 and 56.0 g GAE/mg for Pinot Noir fermented pomace and whole fruit, respectively.

Immediately after drying, freeze dried PN-S and M-S showed the highest ACY of 0.40 and 1.02 mg Mal-3-glu/g d.m., while PN-P and M-P were 0.35 and 0.55 mg Mal-3-glu/g d.m., respectively (Fig. 2). The least ACY was observed in vacuum dry, contained only 45, 60 and 73 % ACY of freeze dried PN-P, PN-S and M-P, respectively. ACY

Table 2. ANOVA table for bioactive compounds of samples during 16 weeks of storage at 15^{o}C

Treatment factors		TPC		ACY		ARS		TFC	
	df	F	P	F	P	F	P	F	P
Drying method	3	33.08	<0.0001	12.69	<0.0001	25.83	<0.0001	22.02	<0.0001
Variety	1	66.55	<0.0001	491.41	<0.0001	21.09	<0.0001	48.11	<0.0001
Byproduct	1	464.88	<0.0001	298.86	<0.0001	642.32	<0.0001	851.74	<0.0001
Storage time	2	135.68	<0.0001	6.44	0.002	98.76	<0.0001	8.62	0.0003
Drying method * Variety	3	2.88	0.376	0.11	0.9544	0.52	0.6673	1.04	0.3793
Drying method * Byproduct	3	4.61	0.004	1.78	0.1526	2.26	0.0832	7.77	<0.0001
Variety * Byproduct	1	33.94	<0.0001	61.38	<0.0001	0.07	0.7903	68.28	<0.0001

TPC = Total phenolics content, ACY = Total anthocyanins content, ARS = Antiradical scavenge activity, TFC = Total flavonal content

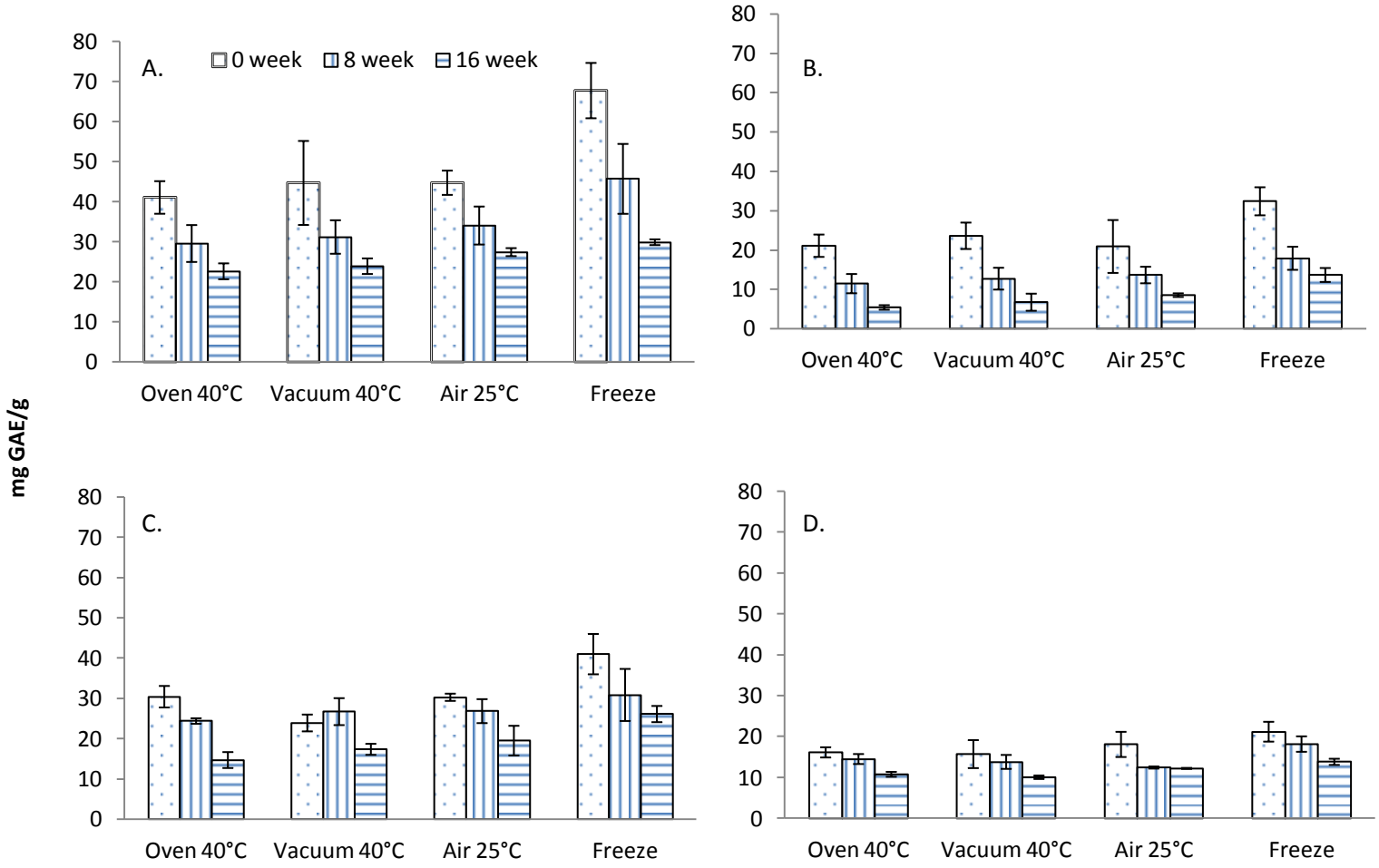

Figure 1. Effect of different drying methods on total phenolic content (TPC) of Pinot Noir pomace (A), Pinot Noir skin (B), Merlot Pomace (C) and Merlot skin (D) immediately after drying and during 16 weeks of storage at 15±2 ^{o}C.

content in Merlot was twice more than in Pinor Noir, and greater concentration of ACY was found in skin than in pomace. deTorres (2010) reported total ACY was 0.34 and 0.27 mg/g d.m. in freeze and 60 °C oven dry, respectively for grape skins based on HPLC analysis, and further identified that malvidin derivatives, malvidin-3-glucoside and malvidin-3-acetylglucoside were the major anthocyanins.

In respect to ARS, freeze dry led to the highest values, in which ARS values of freeze dried PN-P, M-P, PN-S and M-S were 37.46, 34.65, 33.43 and 22.01 mg AAE/g d.m., respectively, immediately after drying (Fig. 3). Air dry retained about 76, 87 and 84% ARS of freeze dried PN-S, M-P and M-S, respectively, while no significant difference in ARS of PN-P samples dried by different methods. ARS in Pinot Noir was significant higher than in Merlot, and pomace had higher ARS than skin. ARS was further expressed as α-tocopherol equilibrium (TE) to meet the requirement for ADF definition. Based on our conversion study (data not shown), 1 mg AAE/g equaled to 2.45 mg TE/g. At week 0, ARS in PN-P, PN-S and M-P samples were all higher than 50 mg TE/g d.m. no matter of the drying method applied, but for M-S samples, only freeze dried one has ARS higher than 50 mg TE/g d.m. The seeds have been demonstrated containing higher amount of oligomeric or polymeric procyanidins, and these compounds had better ability to scavenge the free radicals than monomeric or dimeric procyanidins (Yamakoshi and others 1999).

Immediately after drying, TFC of freeze dried PN-P, M-P, PN-S and M-S was 106.61, 64.38, 30.16 and 37.39 mg CE/g d.m., respectively, showed the highest values compared with oven and air dry (Fig. 4), but no significant difference from vacuum dried samples. Compared to Merlot, Pinot Noir showed higher TFC in pomace, but no difference in skin between the two varieties. Furthermore, TFC in pomace was higher than in skin. Rockenbach (2011) reported that TPC in Pinot Noir were 111.87 mg and 0.56 mg CE/g in seeds and skins, respectively. It was indicated that the great amount of proanthocyanidins, including oligomers and polymers of polyhydroxy flavan-3-ols as (+)-catechin and (-)-epicatechin, exists in the grape seed extracts (Brannan 2008).

Bioactive compounds in WGP were sensitive to heat and can be easily oxidized when subjected to high temperature, while freeze drying at low temperature and vacuum conditions helped retain the bioactive compounds, especially the polyphenolics. Based on

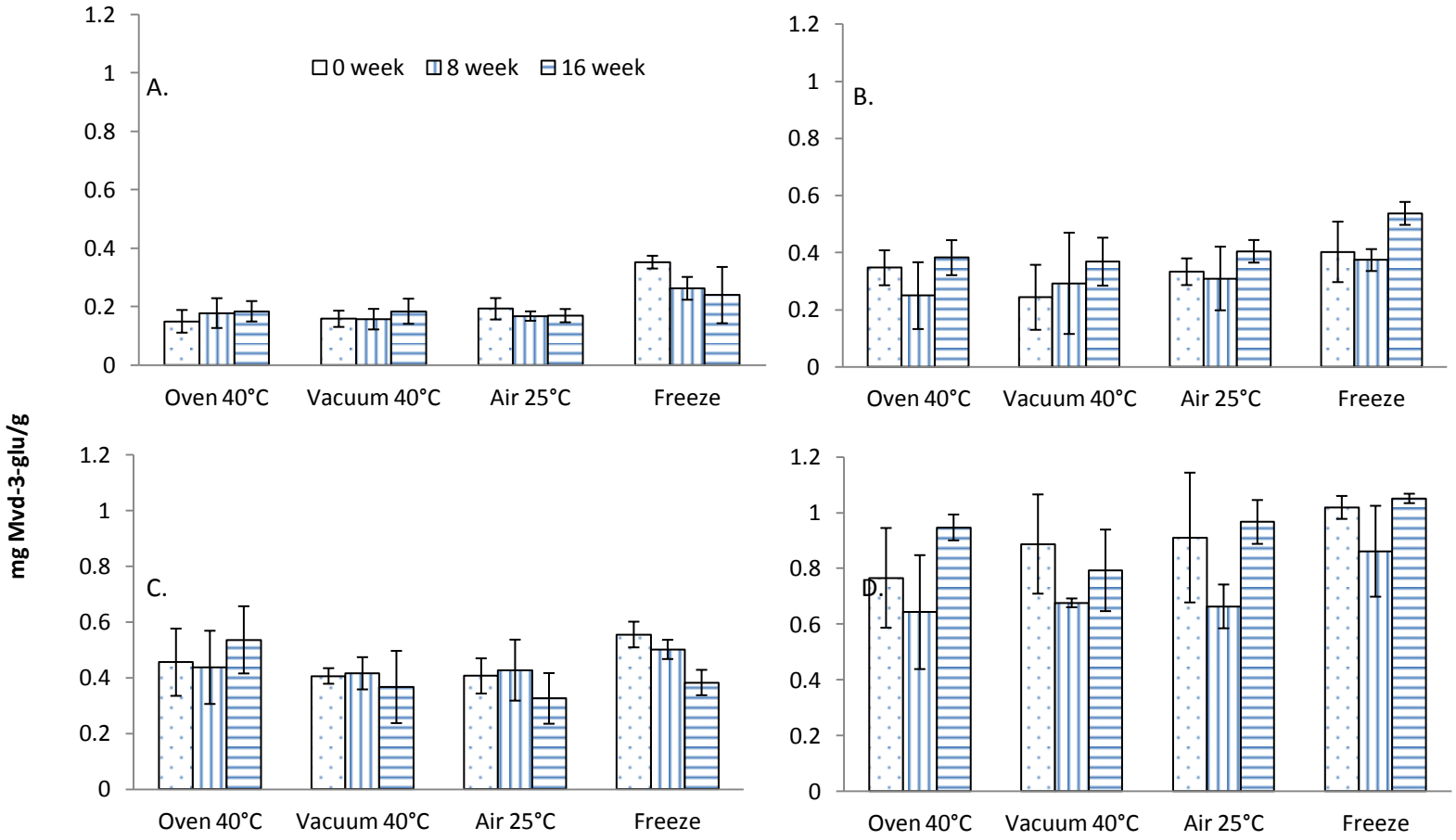

Figure 2. Effect of different drying methods on total anthocyanin content (ACY) of Pinot Noir pomace (A), Pinot Noir skin (B), Merlot pomace (C) and Merlot skin (D) immediately after drying and during 16 weeks of storage at 15±2 ^{o}C.

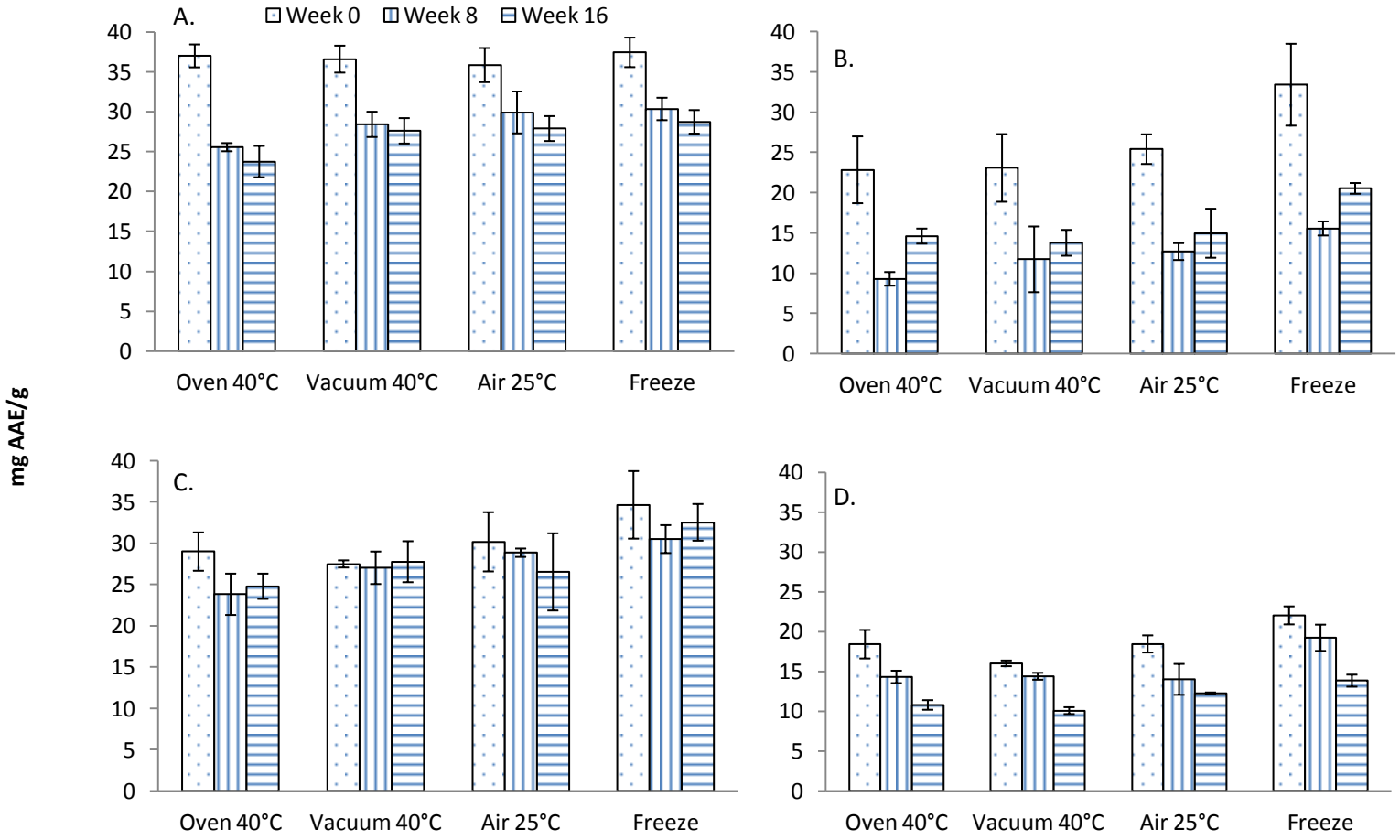

Figure 3. Effect of different drying methods on antiradical scavenge activity (ARS) with ascorbic acid equibilium of Pinot Noir pomace (A), Pinot Noir skin (B), Merlot pomace (C) and Merlot skin (D) immediately after drying and during 16 weeks of storage at 15±2 ^{o}C.

the structure-activity relationships, bioactive compounds may act as free radicals scavenge, chelate metal cations or donate hydrogen atoms or electron to perform the antioxidant activity (Balasundram and others 2006). The antioxidant activity of phenolic acids increased with the increased degree of hydroxylation, which relied on the numbers and positions of the hydroxyl groups within the carboxyl functional group. According to Van Acker and others (1996), gallic acid has trihydroxylate, thus had the highest antioxidant activity among the hydroxybenzoic acids. Both flavanol and anthocyanin were classified as flavonoids with two aromatic rings A and B, joined by a 3-carbon bridge, but different substitution in the form of a heterocyclic ring C. Catechin, one of classification of flavanol, had hydroxylation on carbon 3 at ring C and two -OH groups on the B ring resulted in higher activity and higher stability to the aroxyl radical due to the electron delocalization.

Stability of bioactive compounds during storage

Storage time at 15±2 ^{o}C significantly affected TPC, ACY, ARS and TFC ($P<0.05$) (Table 2). Although freeze dry retained the highest TPC after 16 weeks of storage, the values reduced 56, 58, 36 and 35% for PN-P, PN-S, M-P and M-S, respectively (Fig. 1). TPC reduction rate was the least in air dry, showed 39, 56, 36 and 32% reduction for PN-P, PN-S, M-P and M-S, respectively after 16 weeks of storage. Overall, TPC degradation rate in Pinot Noir were faster than in Merlot for both pomace and skins, and was greater in skins (34% to 74%) than in pomace (27% to 55%) in both varieties.

Among all dried samples, freeze dry retained the highest ACY immediately after drying, but had the least stability during storage at 15±2 ^{o}C. ACY lost 32% and 31% in PN-P and M-P at the end of storage (Fig. 2). There was a trend that ACY of skin samples for both varieties decreased after 8 weeks storage, but increased from 8 to 16 weeks although they were not statically significant. Wang and Stretch (2001) reported the increase in anthocyanin in cranberry aftestored at 15 °C for three months. It may be due to that anthocyanin and non-anthocyanin phenolics are synthesized by the carbon skeletons, provided from decreased titratable acidity and organic acid contents during storage (Mazza 1995).

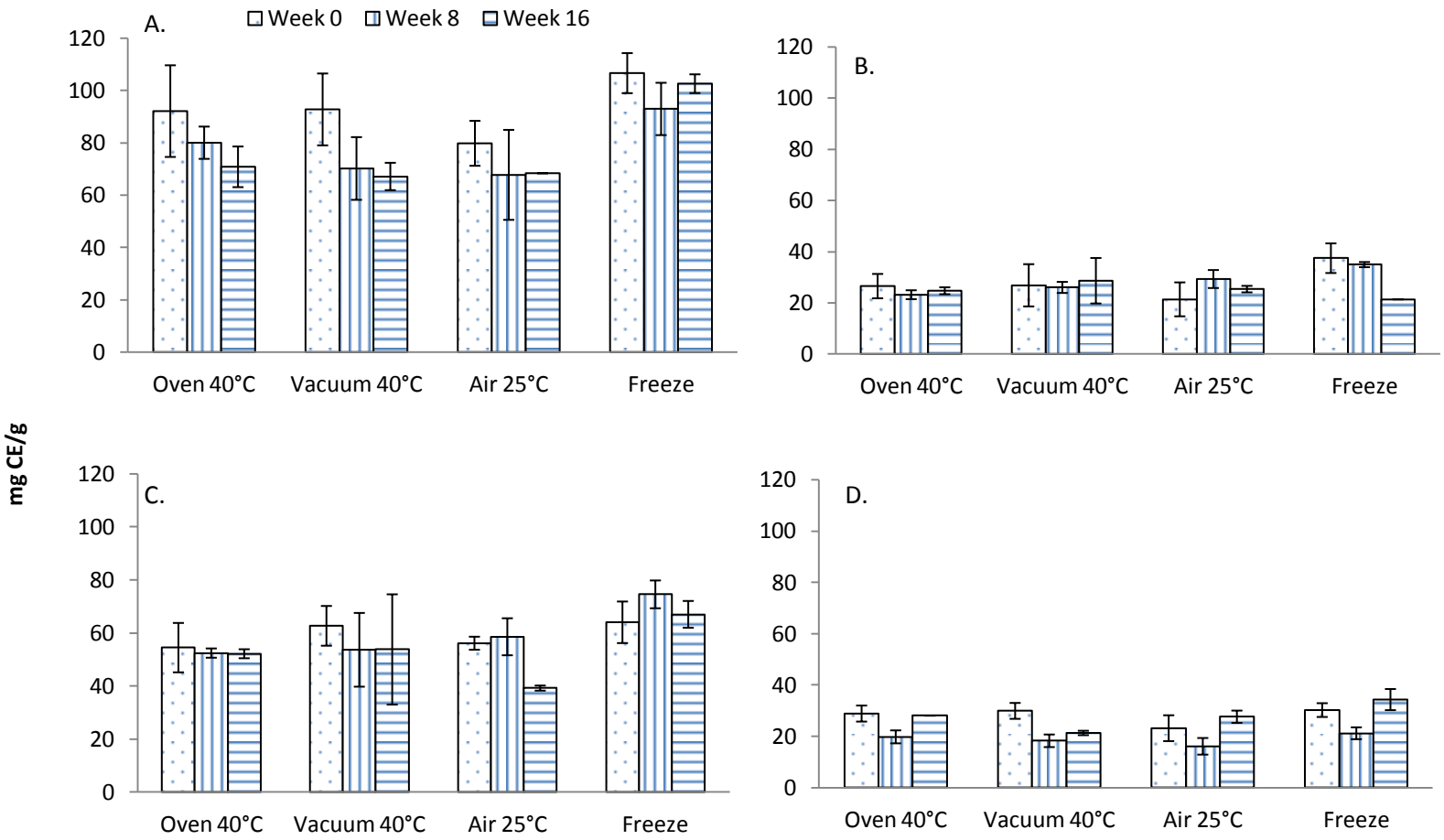

Figure 5. Effect of different drying methods on total flavonol content of Pinot Noir pomace (A), Pinot Noir skin (B), Merlot pomace (C) and Merlot skin (D) immediately after drying and during 16 weeks of storage at 15±2 ^{o}C.

ARS mostly decreased after 8 weeks of storage, but no further change from week 8 to 16, except in M-S (Fig. 3). Freeze dried samples retained the highest amount of ARS after storage with 28.75, 20.54, 32.53 and 20.54 mg/AAE g d.m. in PN-P, PN-S, M-P and M-S, respectively. Air dry had the least ARS degradation rates of 22 and 34% in PN-P and M-S among samples dried by different methods after 16 weeks. ARS in Pinot Noir declined more than in Merlot for both pomace and skin, and skin showed greater ARS degradation than pomace for both varieties. Based on ADF definition, both PN-P and M-P samples met the requirement even after 16 weeks of storage; however, ARS in PN-S and M-S samples were dropped below 50 mg TE/g regardless of the drying methods. Similar ARS reduction trend during storage was found by Medina (2005), who indicated that the radical scavenging capacity by ABTS for pomace-added fish significant decreases during first 2 months, but no further loss at the rest of 6 months of storage.

The stability of TFC varied and no clear trend was observed among different drying methods, between the wine grape varieties and the types of byproduct. Overall, the trend showed, but not statistically significant, that TFC decreased during first 8 weeks of storage, but no reduction on week 8-16, except air dried M-P and freeze dried M-S (Fig. 4). Freeze dried samples lost about 4% and 43% TFC in PN-P and M-S, but increased 14% and 5% in PN-S and M-P at the end of storage. Previous studies reported contradict results on quercetin, one of the flavonol compounds in grape pomace, during fruit storage that quercetin level decreased about 40% in bilberries, but increased 35% in strawberry during 9 months of storage (Careri and others 2003; Hakkinen and others 2000).

Antibacterial activity

Minimum inhibit concentration (MIC) of freeze dried pomace and skin extracts from Pinot Noir and Merlot during 16 weeks of storage are shown in Table 3. The lower MIC indicated the greater antimicrobial activity. Based on our preliminary study (data not shown), there was no significant difference in MIC when adding pomace extract prepared by solvent containing 0.1% HCl. In week 0, MIC was 3% and 4% against *E. coli*, and 2% and 3% against *L. innocua* for PN-P and M-P, respectively. Pinot Noir showed stronger antibacterial activity than Merlot. Also, pomace extracts showed higher inhibition against the growth of *L. innocua* than against *E. coli*. These results were in agreement with

Jayaprakasha (2001) who reported that grape pomace extracts were more effective against Gram-positive bacteria than against Gram-negative, with MIC of 850-1000 ppm and 1250-1500 ppm, respectively. Similar results were also detected by Özkan (2004) that 1% red grape pomace extract against *E. coli* with 6.67 mm inhibition zone.

In respect of skin extracts, MIC was 6% and 9% against *E. coli*, and 7% and 8% for PN-S and M-S against *L. innocua*, respectively. The antibacterial activity of skin extracts followed the same trend as pomace extracts, but MIC values were significantly higher than pomace. As expected, MIC values of pomace and skin extracts significantly increased along with increased storage time. Previous study demonstrated that TPC played a critical role against the growth of microorganisms (Jayaprakasha and others 2003). The bioactive compounds, represented as TPC, were sensitive to temperature and degraded when storage under 15 °C, probably due to the enzymatic activity. Puupponen-Pimiä and others (2001) studied the antimicrobial properties of phenolic compounds from berries and addressed that the degree of hydroxylation might affect the antibacterial activity. The hydroxyl groups on the phenolic compounds interacted with the membrane protein of bacteria by hydrogen bonding that caused the changes in membrane permeability and cell destruction (Boulekbache-Makhlouf and others 2013). Therefore, our results showed a negative correspond ($R^2 > 0.90$) between TPC and MIC against both *L. innocua* and *E. coli* during 16 weeks of storage, except for the PN-S.

Chemical composition and dietary fiber analysis

Chemical compositions of freeze dried samples were reported in Table 4. Moisture content was around 5.18-5.63% which were no significant difference among all the samples. Skin contained significantly higher ash content than pomace, with the highest in PN-S (9.73%) and the lowest in PN-P (5.07%). This explained that most of the minerals were contained in the skins. Protein content was 10.32-11.24%, comparable with previous findings of 12.2-14.39% in red grape skin and pomace. The main protein content was glutamic acid along with limited amount of lysine, tryptophan and sulfur-containing amino acids (Igartuburu and others 1991). The lipid content of pomace was significantly ($P<0.05$) higher than skin, with the highest in PN-P (11.09%) and the lowest in M-S (5.02%). Linoleic acid was found over 80% in fatty acid compounds in grape

Table 3 Minimum inhibit concentration (MIC, expressed as percent of pomace and skin extract) of Pinot Noir and Merlot against *E. coli* and *L. innocua*

MIC (%)	Pinot Noir				Merlot			
	Pomace		Skin		Pomace		Skin	
	E. coli	*L. innocua*	*E. coli*	*L. innocua*	*E. coli*	*L. innocua*	*E. coli*	*L. innocua*
0 week	3%	2%	6%	7%	4%	3%	9%	8%
8 week	6%	5%	8%	8%	6%	5%	11%	11%
16 week	7%	6%	15%	14%	8%	7%	12%	13%
R^{2*}	0.97	0.97	0.67	0.59	0.96	0.96	0.92	0.95

* R^2 indicated the linear relationship between TPC and MIC during 16 weeks of storage at 15±2^{o}C. Pomace = contains wine grape skins and seeds; Skin = wine grape skin only

seeds (Kamel and others 1985; Cao and Ito 2003). Soluble sugar was higher in pomace than in skin and greater in Pinot Noir than in Merlot, with the highest in PN-P (3.89%) and the lowest in M-S (1.20%). The result was consistent with the previous study that the soluble sugar content of white grape seed and skin are 3.02% and 2.71%, respectively (Bravo and Saura-Calixto 1998). Soluble sugar fraction was affected by winemaking process (Llobera and Cañellas 2007). In respect to pectin content, skin contained more pectin than pomace with the highest in M-S (7.63%) and the lowest in PN-P (3.68%). M-S had the highest condensed tannin of 20.96%. Condensed tannin, known as proanthocyanidin, associated with wine astringency during aging process (Gawel 1998).

Table 4 Chemical composition of Pinot Noir and Merlot pomace and skins*

% Composition (DM)	Pinot Noir		Merlot	
	Pomace	Skin	Pomace	Skin
Moisture Content	5.63 ± 0.10^{a}	5.39 ± 0.36^{a}	5.39 ± 0.38^{a}	5.18 ± 0.15^{a}
Ash	5.07 ± 0.05^{c}	9.73 ± 0.53^{a}	6.07 ± 0.50^{b}	9.50 ± 0.25^{a}
Protein	10.32 ± 0.22^{b}	10.67 ± 0.22^{ab}	10.57 ± 0.31^{b}	11.24 ± 0.51^{a}
Lipid	11.09 ± 0.33^{a}	5.11 ± 0.60^{b}	10.47 ± 0.64^{a}	5.02 ± 0.42^{b}
Soluble Sugar	3.89 ± 0.3^{a}	2.07 ± 0.13^{b}	2.11 ± 0.11^{b}	1.20 ± 0.16^{c}
Pectin	3.68 ± 0.05^{c}	6.41 ± 0.52^{ab}	5.82 ± 0.81^{b}	7.63 ± 0.50^{a}
Condensed Tannin	12.11 ± 1.17^{b}	14.46 ± 0.88^{b}	11.66 ± 1.94^{b}	20.96 ± 0.44^{a}
Total Phenolic	6.77 ± 0.70^{a}	3.23 ± 0.36^{c}	4.10 ± 0.50^{b}	2.12 ± 0.19^{d}
Dietary Fiber	61.32 ± 1.69^{a}	55.10 ± 2.79^{b}	57.63 ± 1.56^{ab}	60.00 ± 1.85^{ab}

* Means followed by the same lowercase letters (a – d) in the same row within each varieties and byproducts were not significantly different ($P > 0.05$). Pomace = contains wine grape skins and seeds; Skin = wine grape skin only

Dietary fiber (DF) was the predominate part of wine grape byproduct. Hence, individual fractions of dietary fiber were determined (Table 5). Total DF content was about 61, 55, 58 and 60% in freeze dried PN-P, PN-S, M-P and M-S, respectively, all over 50% dry matter as required in the definition of ADF. SDF was 1.23-1.84% and was only about 2-3% of TDF, in which UA showed greater concentration in skin than in pomace with the highest amount in M-S (0.94%) and the lowest in PN-P (0.35%). IDF

took part of about 97-98% of TDF. KL including ash and resistant protein were higher in pomace than in skin in both varieties with about 45, 40, 36 and 36 % in PN-P, M-P, P-S and M-S, respectively. On the other hand, UA and NS in IDF fraction were found higher in skin. UA in IDF was considered as the pectin bound to cell wall polysaccharide, whereas glucose was the most abundant in NS in IDF by HPLC, which represented as the cellulose and hemicelluloses in grape (Bravo and Saura-Calixto 1998). Therefore, we may conclude that the wine grape byproduct, no matter containing seeds or not, are both good sources of DF.

Conclusion

This study characterized the stabilities of bioactive compounds in two predominant red wine grape pomaces in US northwest region when subjected to different drying methods and storage at 15±2 ^{o}C. Although freeze dry retained higher amount of TPC than ambient air dry, 40^{o}C oven and vacuum dry initially, the difference in other measured bioactive compounds were not significant. Overall, 40°C oven and ambient air dry are highly acceptable by considering the amount of retention of most measured bioactive compounds and their much less cost compared with freeze dry, thus may be employed in commercial application of drying large quantity of wine processing byproducts.

TPC, ARS and TFC were higher in Pinot Noir than in Merlot, and higher in pomace than in skin. Reverse result was observed in ACY. Pomace extract was more efficiency against *L. innocua* than *E. coli*, and showed as a stronger antibacterial agent than skin sample. Pomace contained more lipid, soluble sugar and phenlic compounds than skin samples which had greater concentration of ash and pectin. TDF contents in all samples were more than 50% on a dry matter basis. Specifically, UA and NS in IDF were higher in skin than in pomace, while KL was opposite. Based on our results, Pinot Noir and Merlot pomace can be considered as antioxidant dietary fiber to be used as functional ingredient incorporated into various food products for promoting human health.

Acknowledgement

The authors appreciate the financial support of USDA Center for Small Fruit Research program on this project.

Table 5 Dietary fiber content of Pinot Noir and Merlot pomace and skins

% Dietary Fiber (DM)		Pinot Noir		Merlot	
		Pomace	Skin	Pomace	Skin
Insoluble Dietary Fiber	Uronic Acid	3.21 ± 0.40^{b}	4.23 ± 0.55^{a}	3.31 ± 0.41^{b}	4.71 ± 0.46^{a}
	Neutral Sugar	11.22 ± 0.43^{c}	13.04 ± 0.83^{b}	13.34 ± 0.93^{b}	17.58 ± 0.21^{a}
	Klason Lignin	45.45 ± 1.21^{a}	36.41 ± 1.30^{b}	39.75 ± 1.14^{b}	35.81 ± 0.98^{b}
	Resistant Protein*	7.63 ± 0.55^{c}	9.64 ± 0.49^{b}	7.02 ± 0.46^{c}	10.85 ± 0.90^{a}
	Sum of IDF	59.88 ± 1.64^{a}	53.68 ± 2.68^{b}	56.40 ± 2.48^{ab}	58.15 ± 1.65^{ab}
Soluble Dietary Fiber	Uronic Acid	0.35 ± 0.04^{c}	0.62 ± 0.08^{b}	0.50 ± 0.07^{bc}	0.94 ± 0.11^{a}
	Neutral Sugar	1.09 ± 0.01^{a}	0.80 ± 0.03^{c}	0.73 ± 0.01^{d}	0.91 ± 0.09^{b}
	Sum of SDF	1.44 ± 0.05^{b}	1.42 ± 0.11^{b}	1.23 ± 0.08^{c}	1.85 ± 0.20^{a}
Total Dietary Fiber		61.32 ± 1.69^{a}	55.10 ± 2.79^{b}	57.63 ± 1.56^{ab}	60.00 ± 1.85^{ab}

* Resistant protein was regarded as partial of Klason Lignin and was not calculated in Sum of IDF. Means followed by the same lowercase letters (a–d) in the same row within each varieties and byproducts were not significant different ($P > 0.05$). Pomace = contains wine grape skins and seeds Skin = wine grape skin only

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Wine Grape Pomace as Antioxidant Dietary Fiber for Enhancing Nutritional Value and Improving Storability of Yogurt and Salad Dressing

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ABSTRACT

Wine grape pomace (WGP) as a source of antioxidant dietary fiber (ADF) was fortified in yogurt (Y), Italian (I) and Thousand Island (T) salad dressings. During the three weeks of storage at 4 °C, viscosity and pH of WGP-Y increased and decreased, respectively, but syneresis and lactic acid percentage of WGP-Y and pH of WGP-I and WGP-T were stable. Adding WGP resulted in 35-65% reduction of peroxide values in all samples. Dried whole pomace powder (WP) fortified products had dietary fiber content of 0.94-3.6% (w/w product), mainly insoluble fractions. Total phenolic content and DPPH radical scavenging activity were 958-1340 mg GAE/kg product and 710-936 mg AAE/kg product, respectively. The highest ADF were obtained in 3%WP-Y, 1%WP-I and 2%WP-T, while 1%WP-Y, 0.5%WP-I and 1%WP-T were mostly liked by consumers based on the sensory study. Study demonstrated that WGP may be used as a functional food ingredient for promoting human health and extending shelf-life of food products.

Key words: antioxidant dietary fiber, wine grape pomace, yogurt, salad dressing, storability

Highlights

1. Wine grape pomace was fortified in yogurt and salad dressing.
2. Fortified products had increased dietary fibre and polyphenol contents.
3. Fortified products had delayed lipid oxidation during refrigeration storage.
4. Fortified products were acceptable by consumers based on sensory study.

1. Introduction

The concept of antioxidant dietary fiber (ADF) was first proposed by Saura-Calixto (1998) with the criteria that one gram of ADF should have DPPH free radical scavenging capacity equivalent to at least 50 mg vitamin E and dietary fiber content higher than 50% dry matter from the natural constituents of the material. Wine grape pomace (WGP), the residual seed and skins from winemaking, contain high phenolic compounds and dietary fiber (Deng, Penner & Zhao, 2011; Llobera & Cañellas, 2007). Our previous study found that WGP met the definition of ADF even after 16 weeks of storage under vacuum condition at 15 °C (Tseng & Zhao, 2012). Jiménez et al. (2008) also found that fibers from grapes show higher reducing efficacy in lipid profile and blood pressure than that from oat fiber or psyllium due to combined effect of dietary fiber and antioxidants. WGP as ADF not only retarded human low-density lipoprotein oxidation *in vitro* (Meyer, Jepsen & Sorensen, 1998), but also helped enhance the gastrointestinal health of the host by promoting a beneficial microbiota profile (Pozuelo et al., 2012).

There are increasing interests in applying fruit processing wastes as functional food ingredients since they are rich source of dietary fiber, and most of the beneficial bioactive compounds are remained in those byproducts (Balasundram, Sundram & Samman, 2006). ADF may be incorporated with flour for making high dietary fiber bakery goods, while the polyphenols in ADF could contribute as antioxidant for improving color, aroma and taste of the product. For instance, mango peel powders were used for preparing macaroni to enhance the antioxidant properties (Ajila, Aalami, Leelavathi & Rao, 2010). Apple pomace was incorporated into wheat flour as fiber source to improve the rheological characteristics of cake (Sudha, Baskaran & Leelavathi, 2007). Grape pomace was mixed with sourdough for rye bread (Mildner-Szkudlarz, Zawirska-Wojtasiak, Szwengiel & Pacyński, 2011) and grape seed flour for cereal bars, pancakes and noodles (Rosales Soto, Brown & Ross, 2012).

Aside from promoting human health, WGP as ADF plays important role as antioxidant and antimicrobial agent to extend the shelf-life of food product. For example, WGP was added into minced fish and chicken breast to delay the lipid oxidation (Goni, Sayago-Ayerdi, Brenes & Viveros, 2009; Sánchez-Alonso, Jiménez-Escrig, Saura-Calixto & Borderías, 2007). Also, WGP extract exhibited antimicrobial effect against

foodborne pathogens when added into beef patties (Sagdic, Ozturk, Yilmaz & Yetim, 2011). Research has indicated that WGP seed extracts show better antioxidant activities than that of synthetic antioxidant of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Baydar, Ozkan & Yasar, 2007).

Yogurt is the most popular fermented dairy product with high nutritional value, but not being considered as a significant source of polyphenols and dietary fibers. Fruit are commonly blended in after milk is fermented to make stirred yogurt that is non-Newtonian with weak viscoelastic property (Lubbers, Decourcelle, Vallet & Guichard, 2004). The effects of different types of fruit as source of dietary fiber on the rheological properties of yogurt have been studied (Sendra, Kuri, Fernández-López, Sayas-Barberá, Navarro & Pérez-Alvarez, 2010), and showed stable physicochemical properties of fortified yogurt during storage (Staffolo, Bertola, Martino & Bevilacqua, 2004). A few studies also reported good stability of the bioactive compounds from grape and other plant extract in fortified yogurt (Karaaslan, Ozden, Vardin & Turkoglu, 2011; Wallace & Giusti, 2008).

Salad dressing containing high amount of fat with oil-in-water emulsions can be readily oxidized during processing and storage, which led to the formation of undesirable volatile compounds (Shahidi & Zhong, 2005). Previous studies had added antioxidants to inhibit the lipid oxidation, such as honey (Rasmussen, Wang, Leung, Andrae-Nightingale, Schmidt & Engeseth, 2008), ascorbyl palmitate, α-tocopherol, and ethylenediaminetetraacetic acid (EDTA) (Let, Jacobsen & Meyer, 2007). Orange pulps were also incorporated into salad dressing for enhancing the rheological property and improving storability (Chatsisvili, Amvrosiadis & Kiosseoglou, 2012).

The objective of this study was to investigate the feasibility of fortifying WGP as the source of dietary fiber and polyphenols, i.e., ADF in yogurt and salad dressing for enhancing nutritional value and improving storability of the products. Three different forms of WGP were evaluated, including dried whole grape pomace (WP), pomace liquid extract (LE) and freeze dried liquid extract (FDE). Dietary fiber content were determined for all products, and the quality parameters of fortified products, including pH, peroxide value, total phenolic contents and antiradical scavenging activity were monitored during the refrigeration storage at 4 ^{o}C. Yogurt was further analyzed for viscosity, syneresis and

lactic acid percentage. Moreover, consumer acceptance of WGP fortified yogurt and salad dressing was evaluated through a consumer sensory study. Based on our best knowledge, no study has reported the use of WGP in yogurt and salad dressing and how it may impact the quality of the products.

2. Materials and Methods

2.1 Preparation of wine grape pomace ingredients

The red wine grape pomace (WGP), *Vitis vinifera* L. cv. Pinot Noir, was obtained from the Oregon State University Research Winery (Corvallis, OR, USA). Stems were manually removed to collect seeds and skins. WGP was freeze-dried under -55 °C and vacuum of 17.33 Pa (Model 651 m-9WDF20, Hull Corp., Hatboro, PA) till no further weight loss was observed. Dried WGP was then ground (Gien Mills Inc., NJ) and passed through different sizes of sieves to obtain powders with particle size of 0.85 mm for the analysis of chemical composition and bioactive compounds, and with particle size of 0.18 mm for the fortification in yogurt and salad dressings. Based on our preliminary studies, particle size of WGP directly impacted the sensory quality of fortified products, especially the mouth feeling of fortified yogurt (data not shown). Hence, smaller particle size of 0.18 mm was selected for the fortification.

For preparing the liquid extracts for fortification, WGP powders were extracted by 70% acetone at a solvent to WGP powder ratio of 4:1 (v/w) and ultrasonicated (Branson B-220H, SmithKline Co., Shelton, CT, USA) at room temperature for 60 min. The mixture was centrifuged (International Equipment Co., Boston, MA) at 10,000 g for 15 min and repeated for three times. All supernatants were combined and concentrated by rotation evaporator (Brinkmann Instruments, Westbury, NY, USA) at 40 °C to remove acetone and obtain the WGP liquid extract (LE). The liquid extract was further freeze-dried to obtain freeze-dried pomace extract (FDE). The yield rate of LE and FDE from WGP were about 279% and 8%, respectively. In this study, three forms of WGP, including dried whole powders (WP), LE and FDE, were evaluated for their fortifications in yogurt and salad dressing.

2.2 Chemical composition of WGP

Moisture, ash, protein, fat, condensed tannin and pectin contents of WGP were determined by AOAC methods (Tseng et al., 2012). Dietary fiber (DF), including soluble (SDF) and insoluble dietary fiber (IDF) fractions, was analyzed by the enzymatic-gravimetric method (AOAC 994.13) with some modifications (Deng et al., 2011). In brief, pomace were treated with protease (P-5459, Sigma Chemical Co., USA) in 0.05 M, pH 7.5 phosphate buffer at 60 ℃ for 30 min and then centrifuged. IDF was obtained from the residues, while SDF was supernatant.

SDF fraction was dialysized in deionized water by the tubing with a molecule weight cutoff of 12,000-14,000 (Spectrum Laboratories, Inc., USA) for 48 h. The dialysate was freeze-dried and hydrolyzed with 72% sulfuric acid at 121 ℃ for 1 h. Neutral sugar (NS) was determined based on the anthrone method as D-glucose (Sigma Chemical Co., USA) equivalent. Uronic acid (UA) was quantified by using galacturonic acid (Spectrum Chemical, Co., USA) as standard along with spectrometric assay (UV160U, Shimadzu, Japan). After mixing, 98% H_2SO_4 and boric acid-sodium chloride was incubated at 70 ℃ for 40 min, the solvent was then treated with 3,5-dimethyphenol-glacial acetic acid (Sigma Chemical Co., USA) and the absorbance was measured at 400 and 450 nm, respectively. SDF was calculated as sum of NS and UA.

IDF fraction was hydrolyzed by 72% sulfuric acid at 30 ℃ for 1 h, followed at 121 ℃ for 1 h. The mixture was filtrated by fritted crucible, in which the filtrate was used for NS and UA measurement as described for SDF, while the residue was considered as Klason lignin (KL) after drying for 16 h at 105 ℃. IDF was quantified by the sum of KL, NS and UA, and total dietary fiber content was calculated as sum of IDF and SDF.

2.3 Total phenolic content and DPPH radical scavenging activity of WGP

WGP was extracted by using 70% acetone /0.1% HCl (v/v) at solvent/pomace powder ratio of 4:1 (v/w) (Deng et al., 2011) and followed the same procedure as described above in obtaining LE. The final extract was used for determining total phenolic content (TPC) and DPPH radical scavenging activity (RSA).

TPC was measured by the Folin-Ciocalteu assay along with spectrometer. The diluted extract was reacted with Folin-Ciocalteu reagent (Sigma Chemical Co., MO, USA) for 10

min followed with addition of 20% $NaCO_3$ and incubation in a 40 °C water bath for 15 min (UV160U, Shimadzu, Japan). Gallic acid (Sigma Chemical Co., USA) was applied as a standard, and the results were expressed as mg gallic acid equivalent (GAE)/g WGP at absorbance of 765 nm using a spectrometer (UV160U, Shimadzu, Japan).

RSA was determined by 1,1-diphenyl-2-picryhydrazyl (DPPH) (Kasel Kogyo Co. Ltd, Japan) assay based on ascorbic acid (Mallinckrodt Baker Inc., USA) equivalent. The diluted extract was mixed with DPPH-methanol reagent (9 mg DPPH in 100 mL methanol) for 10 min at room temperature and the absorbance was read at 517 nm. The results were expressed as mg ascorbic acid equivalents (AAE)/g WGP.

2.4 Preparation of yogurt and salad dressing

Yogurt was prepared using reduced fat milk (2% milk fat, Darigold, USA) with 4% sugar (w/v milk) addition. Sugar was dissolved in the milk and pasteurized in 85 ºC water bath for 30 min and then cooled down to 45 ºC. Starter culture (ABY 2C, Dairy Connection Inc., Wisconsin, USA), a combination of *Streptococcus thermophiles, Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis* was added. The mixture was fermented in a 45 ºC water bath till the final pH of 4.5 (about 4.5 h). After the milk was coagulated, 1, 2, or 3 g WP was added to make 100 g yogurt and stirred gently, named as 1%, 2% and 3% WP (w/w yogurt), respectively. Based on our preliminary study, 2% WP (w/w yogurt) sample obtained the best overall physicochemical properties and stability during storage. The amount of LE and FDE added into yogurt was then calculated to achieve approximate same amount of TPC as that in 2% WP. Hence, 5.59 mL LE and 0.215 g FDE were added into 100 g of yogurt and named LE-Y and FDE-Y, respectively. Yogurt samples were packed into polyethylene bottle (Dynalab Corp., NY, USA) and stored at 4 ºC refrigerator under dark for quality evaluation at day 1 (overnight), 7, 14 and 21.

Two types of commercial salad dressing were purchased from a local grocery store, Italian and Thousand Island (Kraft, USA), representing the liquid and creaming type, respectively. Based on our preliminary study on the texture and visual appearance of WP fortified dressing, 0.5 g and 1 g of WP (named 0.5% WP and 1% WP (w/w Italian), respectively), 2.795 mL LE (named LE-I) and 0.1075 g FDE (named FDE-I) were added

into 100 g of Italian dressing, while 1 g and 2 g of WP (named 1% WP and 2% WP (w/w Thousand Island), respectively), 5.59 mL LE (named LE-T) and 0.215 g FDE (named FDE-T) were incorporated into 100 g of Thousand Island. WGP fortified salad dressings were stored at the same 4 ºC refrigerator for quality evaluation at day 0, 7, 14, 21 and 28.

2.5 Color and pH of WGP fortified yogurt and salad dressings

Color of the samples was monitored by a colorimeter (Lab Scan II, Hunter Associate Laboratory Inc., Reston, VA, USA). Samples were placed inside a glass refract cup on the light pore size of 44.45 mm. Data were recorded as CIE L* values indicating lightness, as well as Chroma value of $(a^2+b^2)^{1/2}$ and Hue angle of $\tan^{-1}(b/a)$ to represent the saturation and shade of the color, respectively. The pH of the samples was measured by a pH meter (Corning, NY, USA).

2.6 Syneresis, viscosity, and lactic acid percentage of WGP fortified yogurt

Syneresis is defined as whey separation from gel matrix and considered as an important quality indicator of yogurt. To determine syneresis, 20 g of yogurt was spread as a thin layer on the Whatman No.1 filter paper and vacuum drained by a Buchner funnel. Syneresis was calculated as the percentage of whey loss by the total sample. Viscosity of the yogurt was measured by a rotational viscometer (DV-III, Brookfield, MA, USA) with spindle No. 93 at the speed of 25 rpm, and recorded as centipoises (cP). Lactic acid percentage was determined by titration with standard 0.1 N NaOH until reaching pH 8.2.

2.7 Peroxide value of WGP fortified yogurt and salad dressings

Peroxide value (PV) was expressed as the amount of peroxides formed in oils and fats during oxidation and was measured by the acetic acid-chloroform method (AOCS Cd 8-53). In brief, 2 g of sample was homogenized with 30 mL of acetic acid: chloroform at 3:2 (v/v) and filtrated by Whatman No.1 filter paper. Filtrate was added with 0.5 mL saturated potassium iodine and occasionally shaken for 1 min. Thirty mL of water was then added, and the mixture was titrated with 0.01 N standard sodium thiosulfate until transparent. The results were expressed as milliequivalent peroxide/kg product.

2.8 Total phenolic compound, DPPH radical scavenging activity and dietary fiber of WGP fortified yogurt and salad dressings

To extract the bioactive compounds in WGP fortified yogurt and salad dressings, a 20 g of sample was mixed with 30 mL 70% acetone /0.1% HCl (v/v) and set at 4 ℃ overnight. Solution was then passed through filter paper (Whatman No.1) to collect the filtrate, and concentrated using a rotation evaporator at 40 ℃. TPC and RSA were quantified by the same procedures for WGP described above (section 2.3), and the results were express as mg GAE/kg and mg AAE/kg product, respectively. For DF analysis, samples were washed with petroleum ether twice under ultrasonication and then followed the steps as described above for WGP determination. The results were expressed as TDF, IDF and SDF percentage of product. The commercial fiber-added yogurt (FiberOne with blueberry, YoPlait, USA) was set as reference, and its TPC, RSA and DF were determined right after purchase, while TPC and RSA of WGP fortified yogurt and salad dressings were measured during 3 and 4 weeks of storage at 4 ℃, respectively.

2.9 Sensory evaluation of WGP fortified yogurt and salad dressings

Permission of the sensory study was obtained from the Institutional Review Board at the Oregon State University. Panelists were recruited by E-mails and screened to meet the requirement of consuming flavored yogurt or salad with dressing more than 3 times a week. Twelve panelists (age between 18 and 39, 4-5 males and 7-8 females depending on the type of product tested) were participated in the sensory evaluation of each product. Only products fortified with WP were evaluated for consumer sensory acceptance since WP provides the highest amount of ADF. Commercial vanilla flavor plain yogurt (YoPlait Original, USA) mixed with 5.59% grape juice concentrate (v/w yogurt) (Albertson, USA) was used as a control to avoid the discrimination in color and flavor. Salad dressings were served with field green salad (Dole, USA), by giving instruction to the panelists to pour the dressings on the salad based on their preferred amount. Panelists were asked to rate the likeness on appearance, overall, flavor and texture quality of the samples by using a 9-point hedonic scale (9=like extremely, 1=dislike extremely). The consistency of the products were evaluated by ‘Just About Right’ scale (5=too thick,

1=too thin, and 3= just about right). An open-end question was also asked at the end to describe the reasons for liking and disliking the products.

2.10 Data analysis

All the experiments, except the sensory evaluation, were conducted triplicate and the mean values were compared based on LSD at 95% confidence level. For storage study, the analysis of variance (ANOVA) was performed to evaluate significant treatment effect of two independent factors: WGP forms (different WP concentrations, LE and FDE) and storage time. All data were analyzed by general linear model procedure (PROC GLM) of SAS 9.2 (SAS Inst. Inc., USA). For sensory evaluation, the results were exported from Compusense Programme (Compusense 5.0, version 4.6, Guleph, Canada), and the means of consumer acceptance results for each attribute were analyzed by ANOVA and compared at the $P<0.05$ level by Tukey test.

3. Results and Discussions

3.1 Chemical composition of WGP

Fat, protein, soluble sugar, pectin and condensed tannin content of WGP were 11.09, 10.32, 3.89, 3.68 and 12.11%, respectively (Table 1), comparable to the data in previous study (Llobera et al., 2007). TPC of WGP was 67.74 mg GAE/g. Note that phenolic compounds in WGP are influenced by many factors, including grape variety, growth climate and location, harvest time, as well as processing and storage conditions, extraction and analytical methods (Lafka, Sinanoglou & Lazos, 2007). Thimothe, Bonsi, Padilla-Zakour and Koo (2007) reported that Pinot Noir pomace after fermentation in winemaking has slightly higher TPC than that of whole Pinot Noir fruit. In general, phenolic acids including gallic acid and ellagic acid, and flavonoids, such as catechin, epicatechin, procyanidins and anthocyanins are the major polyphenols in WGP (Lafka et al., 2007; Yilmaz & Toledo, 2006). Lu and Foo (1999) detected 17 polyphenols in WGP and Schieber, Kammerer, Claus & Carle (2004) further quantified 13 anthocyanins, 11 phenolic acids, 13 flavonoids, and 2 stilbenes in WGP by HPLC. Anthocyanin contributed to the color of the WGP was identified as malvidin derivatives, malvidin-3-glucoside and malvidin-3-acetylglucoside (de Torres, Díaz-Maroto, Hermosín-Gutiérrez

& Pérez-Coello, 2010). Phenolic compounds are the secondary metabolites of plants and characterized by the structure-activity relationship of the hydroxyl group and the nature of substitutions on aromatic ring. Based on their structure-activity relationship, there are several different antioxidant mechanisms of phenolics, such as free radicals scavenging ability, hydrogen atoms or electron donation and metal cations chelation (Amarowicz, Pegg, Rahimi-Moghaddam, Barl & Weil, 2004).

Total DF content of WGP was about 61%, met the definition of ADF with over 50% dry matter. In respect to RSA, 1 mg AAE/g equaled to 2.45 mg α-tocopherol equilibrium (TE)/g based on our previous study (Tseng et al., 2012). RSA of WGP was 37.46 AAE/g or 91.78 TE/g, also met the requirement for ADF of having free radical scavenging at least equivalent to 50 mg of vitamin E by DPPH method. These properties are intrinsic to the WGP, deriving from the natural constituents of the material. Additionally, WGP retained the ADF characteristic even after 16 weeks of storage at 15 °C in vacuum package (Tseng et al., 2012). Therefore, WGP could be claimed as antioxidant dietary fiber and fortified in yogurt and salad dressings in this study.

Table 1

	% Composition (DM) *
Moisture Content	5.63 ± 0.10
Ash	5.07 ± 0.05
Protein	10.32 ± 0.22
Lipid	11.09 ± 0.33
Soluble Sugar	3.89 ± 0.3
Pectin	3.68 ± 0.05
Condensed Tannin	12.11 ± 1.17
Dietary Fiber	61.32 ± 1.69
Total Phenolic Compound (mg GAE/g)	67.74 ± 6.91
Radical Scavenge Activity (mg AAE/g)	37.46 ± 1.86
Radical Scavenge Activity (mg TE/g)	91.78 ± 4.58

Chemical composition, total phenolic content and DPPH radical scavenging activity of wine grape pomace (WGP)
* DM = dry matter. The table was modified from the Tseng & Zhao (2012).

3.2 Color of WGP and WGP fortified yogurt and salad dressings

L*, Hue and Chroma values of freeze dried WGP and its fortified products are presented in Table 3. The control yogurt sample without the addition of WGP received the highest L* of 92.18, but the lowest Hue value of -1.26. As expected, the lightness and Hue values decreased, but the Chroma increased along with increased amount of WP added, but no significant difference ($P<0.05$) between 2% WP and 3% WP (w/w yogurt) samples. Overall, LE-Y and FDF-Y samples obtained the higher ($P<0.05$) L* and Hue values, but lower Chroma value than those of 2% WP (w/w yogurt) sample. These results reflected that the LE and FDE fortified samples provide more homogeneous but less saturated color in the product. Also, WP presented more redness and blueness compared to LE and FDE that showed higher a* value, but lower b* value (data not shown).

In respect to WGP fortified salad dressings, the control sample received the lightest color, 43.59 and 72.25 in Italian and Thousand Island dressing, respectively; while the darkest color was found in 1% WP (w/w Italian) (36.96) and 2% WP (w/w Thousand Island) (60.33) samples. In Italian dressing, the lowest Hue value was found in LE-I (1.09), but no difference ($P>0.05$) among all Thousand Island samples regardless of the concentration and type of WGP added. Both Italian and Thousand Island samples had the high Chroma value of 29.06 and 39.47, respectively, and the samples with the highest amount of WGP received the lowest Chroma values, 21.79 in 1% WP (w/w Italian) and 28.16 in 2% WP (w/w Thousand Island).

3.3 pH of WGP fortified yogurt and salad dressings

Figure 1 shows the pH of WGP fortified products during 4 weeks of storage under 4 °C. Adding WGP into the yogurt immediately reduced the pH from 4.78 to 4.47-4.60. Since WGP liquid extract had a low pH of 3.63, LE-Y showed the lowest pH of 4.47. The pH of all samples continuously dropped ($P<0.05$) during the first 2 weeks of storage. At the end of 4 weeks, control sample remained the highest pH of 4.44, while LE-Y had pH of 4.30. These results were consistent with previous study in orange fiber fortified yogurt, in which about 0.2 unit of pH reduction was observed after 14 days of storage (García-Pérez, Lario, Fernández-López, Sayas, Pérez-Alvarez & Sendra, 2005). Beal, Skokanova, Latrille, Martin and Corrieu (1999) explained that the high rate of production of lactic

Table 3
Color of wine grape pomace (WGP) and WGP fortified yogurt and salad dressing *

		Lightness	Hue	Chroma
WGP		43.32 ± 0.35	0.73 ± 0.00	15.25 ± 0.23
WGP fortified Yogurt	Control	92.18 ± 0.61 a	-1.26 ± 0.01c	8.11 ± 0.69 b
	1 % WP	79.53 ± 9.89 b	0.93 ± 0.07 a	6.37 ± 0.48 c
	2 % WP	61.68 ± 0.94 c	0.84 ± 0.04 b	9.99 ± 1.18 a
	3 % WP	58.17 ± 1.35 c	0.80 ± 0.05 b	10.46 ± 1.77 a
	LE-Y	83.47 ± 0.25 b	0.96 ± 0.03 a	6.23 ± 0.13 c
	FDE-Y	81.96 ± 0.20 b	0.93 ± 0.02 a	6.86 ± 0.14 bc
WGP fortified House Italian	Control	43.59 ± 0.20 a	1.14 ± 0.01 a	29.96 ± 0.33 a
	0.5 % WP	39.76 ± 0.28 c	1.06 ± 0.05 bc	24.04 ± 2.13 c
	1 % WP	36.96 ± 0.17 d	1.02 ± 0.01 b	21.79 ± 0.35 d
	LE-I	43.39 ± 0.45 a	1.09 ± 0.00 c	26.60 ± 0.16 b
	FDE-I	41.49 ± 0.14 b	1.09 ± 0.01 b	27.43 ± 0.23 b
WGP fortified Thousand Island	Control	72.25 ± 0.17 a	1.10 ± 0.00 a	39.47 ± 0.23 a
	1 % WP	68.04 ± 0.07 c	1.10 ± 0.01 a	34.21 ± 0.43 d
	2 % WP	60.33 ± 0.47 d	1.11 ± 0.02 a	28.16 ± 0.05 e
	LE-T	70.65 ± 0.38 b	1.09 ± 0.02 a	35.40 ± 0.41 c
	FDE-T	71.99 ± 1.16 a	1.10 ± 0.02 a	36.36 ± 0.49 b

* Means followed by the lowercase letters (a - d) in the same column within each concentration of WGP fortified product were not significantly different ($P > 0.05$). Control= no pomace added, WP= whole pomace powder, LE= liquid pomace extract, and FDE= freeze dried pomace extract.

acid and galactose was observed at the initial 14 days due to the high bacterial metabolic activity with the consumption of lactose.

The pH of WGP fortified Italian salad dressing was lower than control initially, but no difference ($P>0.05$) in pH among all fortified samples no matter of the type and concentration of WGP added. The control and WGP fortified samples had pH of 3.41 and ~3.38, respectively at day 0. Overall, the pH was slightly dropped during storage under 4 °C and received the value of 3.35 and 3.31 in control and 1% WP (w/w Italian) samples, respectively at the end of 4 weeks of storage. For Thousand Island salad dressing, 2% WP (w/w Thousand Island) obtained the relatively low pH of 3.53, whereas the control had a pH of 3.57. The pH of LE-T sample was slightly higher, probably due to the higher pH of the extract. The pH of the Thousand Island dressing remained stable, about 3.5 to 3.6 during 4 weeks of storage.

3.4 Syneresis, viscosity and lactic acid percentage of WGP fortified yogurt

Based on our preliminary study, 2% reduced fat milk could not coagulate if >5% WP (w/w yogurt) was added before fermentation. Also, it required longer fermentation time when adding more than 3% WP (w/w yogurt) into milk beforehand, which was undesirable due to increasing in syneresis. Mazaheri, Tehrani and Shahidi (2008) also found that syneresis was lower when fruit were added after fermentation. Therefore, WGP was added after the milk had coagulated, i.e., yogurt had formed in this study.

Viscosity, syneresis and lactic acid percentage of WGP fortified yogurt during 4 weeks of storage at 4°C are reported in Table 4. No difference ($P>0.05$) on syneresis among all the samples was observed initially, ranged from 16.82 to 20.13% (Table 4). The syneresis increased significantly ($P<0.05$) only in 3% WP (w/w yogurt) sample (33.58%), while all other samples remained stable during 3 weeks of storage. The amount of WP addition in yogurt is critical because the protein in WP rearranged the gel matrix. Hence, 2% WP (w/w yogurt) was selected as the optimum level of WGP fortification in yogurt and the same concentration was then applied to select the level of LE-Y and FDE-Y to be added in yogurt. Staffolo and others (2004) reported that no syneresis was occurred when yogurt was fortified with 1.3% of wheat, bamboo, inulin and apple fiber during 21 days of storage.

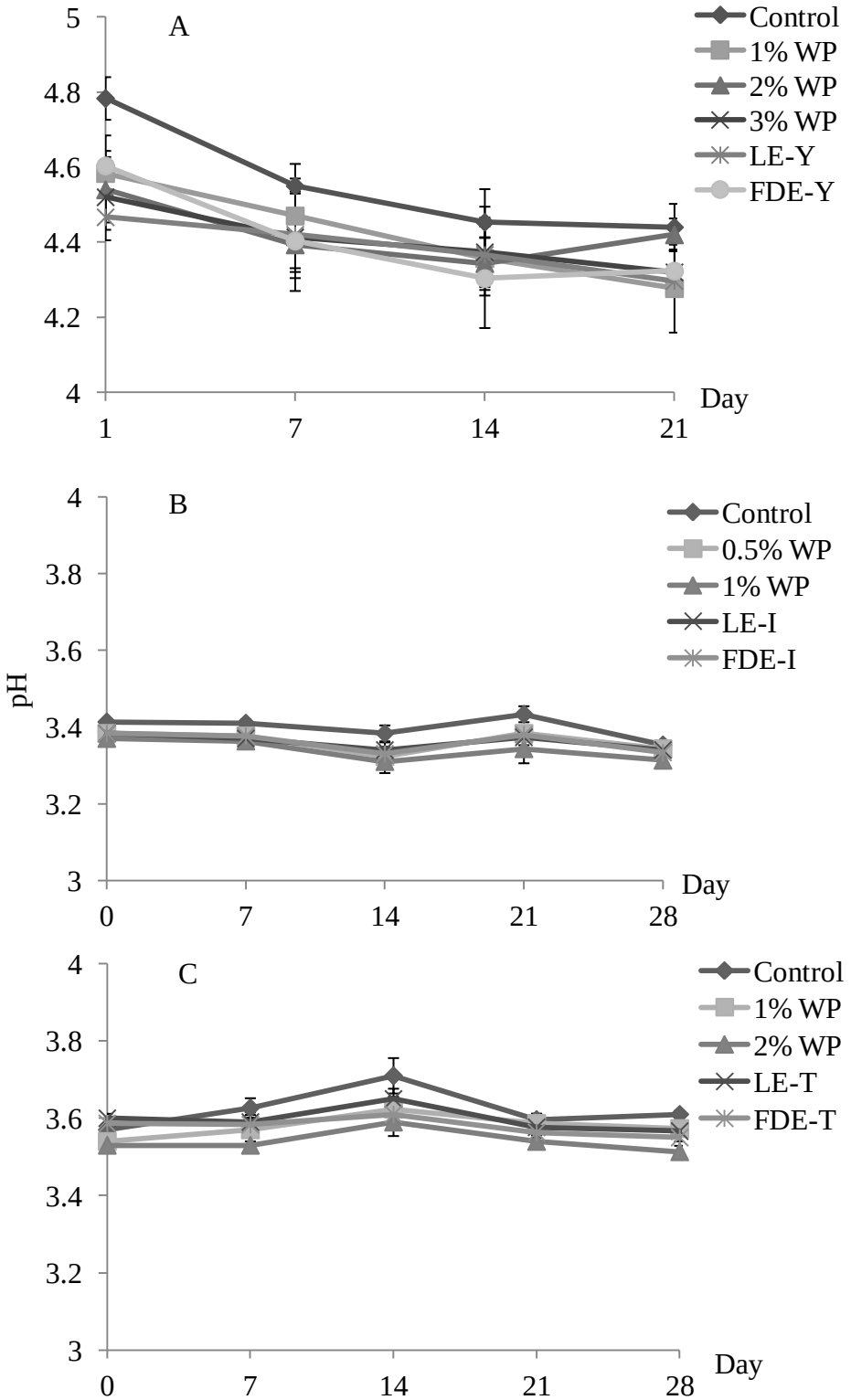

Fig. 1. pH value of samples during storage at 4 °C. (A) WGP fortified yogurt, (B) WGP fortified Italian salad dressing, and (C) WGP fortified Thousand Island salad dressing.

Adding WGP reduced viscosity of yogurt, in which 3% WP (w/w yogurt) sample had the lowest value of 533 cP, while it was 1267 cP in the control (Table 4). This result was probably because stirring high concentration of WP in yogurt broke down the coagulated milk, thus reduced the viscosity. Viscosity of FDE-Y and WP fortified yogurt samples all increased during 3 weeks of storage, in which FDE-Y samples increased from 1533 cP to 3407 cP, and 1% WP, 2%WP and 3% WP (w/w yogurt) samples increased 252, 351 and 428%, respectively, higher than those of control, LE-Y and FDE-Y samples, probably contributed by the insoluble dietary fiber fraction in WP. Ramaswamy and Basak (1992) stated that the addition of WGP or fruit concentrate generally decreased the consistency of the products owning to reduced water-binding capacity of proteins. During the storage time, the increased viscosity could be regarded as recovery of structure or rebodying (Lee & Lucey, 2010). In addition, dietary fiber in WGP may influence the viscosity of the products. Grigelmo-Miguel, Ibarz-Ribas & Martin-Belloso (1999) reported increased viscosity along with the increasing of fiber concentration in yogurt.

WP fortified yogurt obtained relatively higher lactic acid percentage of 0.76 to 0.79% initially, while LE-Y and FDE-Y fortified ones had the lowest value of 0.67 and 0.65%, respectively (Table 4). WP contained some lactic acid may generated during winemaking process, but this organic acid was washed away during extraction in LE and FDE. Overall, lactic acid percentage of WP fortified yogurt increased during 3 weeks of storage except in control, 1% WP, 2% WP and 3% WP (w/w yogurt) samples. At the end of 4 weeks of storage, control sample showed the lowest lactic acid percentage of 0.76 %, while there was no difference ($P>0.05$) among WGP fortified ones, ranging from 0.79% to 0.89%.

3.5 Peroxide value of WGP fortified yogurt and salad dressings

As shown in Figure 2, peroxide value (PV) increased along with storage time, and the control had significantly ($P<0.05$) higher values than those of WGP fortified ones. Control and 1% WP (w/w yogurt) samples started to oxidize within 7 days, while PV in 3% WP (w/w yogurt) was not detectable until almost 14 days. At the end of 3 weeks of storage, 3% WP (w/w yogurt) had the lowest PV of 1.81 meq/kg yogurt, while PV for other WGP fortified yogurt samples was in the range of 2.04 to 2.15 meq/kg yogurt, and

Table 4
Syneresis, viscosity, and lactic acid percentage of wine grape pomace (WGP) fortified yogurt during 3 weeks of storage at 4 °C *

Parameter	Treatment	0 day	7 day	14 day	21 day
Syneresis	Control	A 18.59 ± 2.17 a	BC 25.16 ± 3.85 a	A 25.05 ± 6.56 a	A 19.60 ± 5.81a
	1 % WP	A 17.25 ± 3.67 a	AB 20.10 ± 0.74 a	A 21.21 ± 4.87 a	A 20.49 ± 0.60 a
	2 % WP	A 19.67 ± 3.10 a	BC 23.85 ± 6.00 a	A 22.13 ± 4.12 a	AB 25.49 ± 8.65 a
	3 % WP	A 18.70 ± 3.07 a	C 27.57 ± 5.26 ab	A 27.21 ± 2.87ab	B 33.58 ± 12.99 b
	LE	A 20.13 ± 2.39 a	A 18.47 ± 2.49 a	A 27.08 ± 1.44 a	A 20.94 ± 1.38 a
	FDE	A 16.82 ± 5.57 ab	AB 16.18 ± 3.40 ab	A 23.53 ± 2.39 b	A 15.70 ± 4.14 a
Viscosity	Control	B 1266.67 ± 41.63 c	B 2380.00 ± 346.99 b	AB 2770.00± 710.84 ab	AB 3246.67 ± 141.89 a
	1 % WP	C 613.33 ± 41.63 b	BC 2213.33 ± 162.89 a	B 1860.00 ± 650.23 a	C 2160.00 ± 713.58 a
	2 % WP	C 580.00 ± 72.11 c	C 1874.50 ± 128.34 b	AB 2013.33 ± 498.93 b	BC 2620.00 ± 321.87 a
	3 % WP	C 553.33 ± 23.09 c	C 1940.00 ± 419.05 b	B 1936.67 ± 539.48 b	AB 2924.67 ± 348.35 a
	LE	B 1320.00 ± 72.11 c	AB 2600.00 ± 69.28 ab	AB 2183.33 ± 195.02 b	AB 2913.33 ± 438.79 a
	FDE	A 1533.33 ± 23.09 b	A 2861.67 ± 150.53 a	A 2983.33 ± 739.21 a	A 3406.67 ± 306.16 a
Lactic Acid Percentage	Control	AB 0.73 ± 0.01 a	AB 0.73 ± 0.10 a	BC 0.74 ± 0.05 a	B 0.76± 0.04 a
	1 % WP	A 0.76 ± 0.05 a	AB 0.77 ± 0.04 a	A 0.83 ± 0.07 a	A 0.87 ± 0.07 a
	2 % WP	A 0.79 ± 0.05 a	A 0.82 ± 0.01 a	AB 0.82 ± 0.04 a	A 0.88 ± 0.10 a
	3 % WP	A 0.77 ± 0.07 a	AB 0.78 ± 0.11 a	A 0.85 ± 0.03 a	A 0.89 ± 0.01 a
	LE	B 0.67 ± 0.02 c	B 0.66 ± 0.01 c	C 0.73 ± 0.03 b	AB 0.79 ± 0.03 a
	FDE	B 0.65 ± 0.02 b	AB 0.79 ± 0.04 a	ABC 0.78 ± 0.03 a	AB 0.82 ± 0.02 a

* Means followed by same capital letters (A – D) in same column within each concentration were not significantly different ($P > 0.05$). Means followed by same lowercase letters (a – d) in same row within each storage day were not significantly different ($P > 0.05$). Control= no pomace added, WP= whole pomace powder, LE= liquid pomace extract, and FDE= freeze dried pomace extract.

PV of control was the highest, 7.08 meq/kg yogurt. These results indicated that the amount of WGP played more important role on PV than the form.

PV of the commercial Italian and Thousand Island dressings (control) at the point of purchase were 3.45 and 7.21 meq/kg, respectively. PV of WGP fortified Italian dressing remained stable during 4 weeks of storage, except a slightly increase in 0.5% WP (w/w Italian). At the end of 4 weeks of storage, PV of control was 14.47 meq/kg Italian, while that of 1% WP (w/w Italian), LE-I and FDE-I samples were 2.48, 4.03 and 4.13 meq/kg Italian, respectively, no difference among WGP fortified samples ($P>0.05$). In respect to the Thousand Island samples, PV of control at 4 weeks was 26.62 meq/kg Thousand Island, while that of 2% WP (w/w Thousand Island), LE-T and FDE-T samples were 16.69, 16.93 and 17.36 meq/kg Thousand Island, respectively, again no difference among WGP fortified samples ($P>0.05$). Ifesan et al. (2009) investigated salad dressing fortified with herb *Eleutherine americana* crude extract, and obtained lower thiobarbituric acid reactive substance (TBARS) value and retarded malonaldehyde formation due to the redox properties of antioxidant activity from the extract.

Lipid oxidation is one of the major concerns in food quality deterioration. The oxidative process may be catalyzed by light, heat, enzymes, metals, metalloproteins and microorganisms that lead the development of off-flavor. The formation of hydroperoxides (ROOH) may break down to a variety of nonvolatile and volatile secondary products. PV, represented as the total hydroperoxide content, is an indicator of the initial stages of oxidation and predicts rancidity of a product (Shahidi et al., 2005). No off-odor was detected subjectively in all WGP fortified products during the whole storage based on authors' observation. The phenolic hydroxyl groups in WGP could reduce the PV value and delay lipid oxidation by donating hydrogen atom to scavenge free radicals, such as hydroxyl, peroxyl, superoxide and nitric oxide, and form the stable end product in order to interfering the initiation or propagation for further lipid oxidation (Sánchez-Alonso et al., 2007). WGP extract has been evaluated as safe and natural antioxidant fortified in various food products to inhibit the formation of toxic oxidation products, prevent rancidity in lipid systems and prolong the shelf-life. For examples, WGP extract showed high antioxidant effect in sunflower oil against the formation of secondary oxidation products and stronger antioxidant effect than that of tocopherols in soybean oil

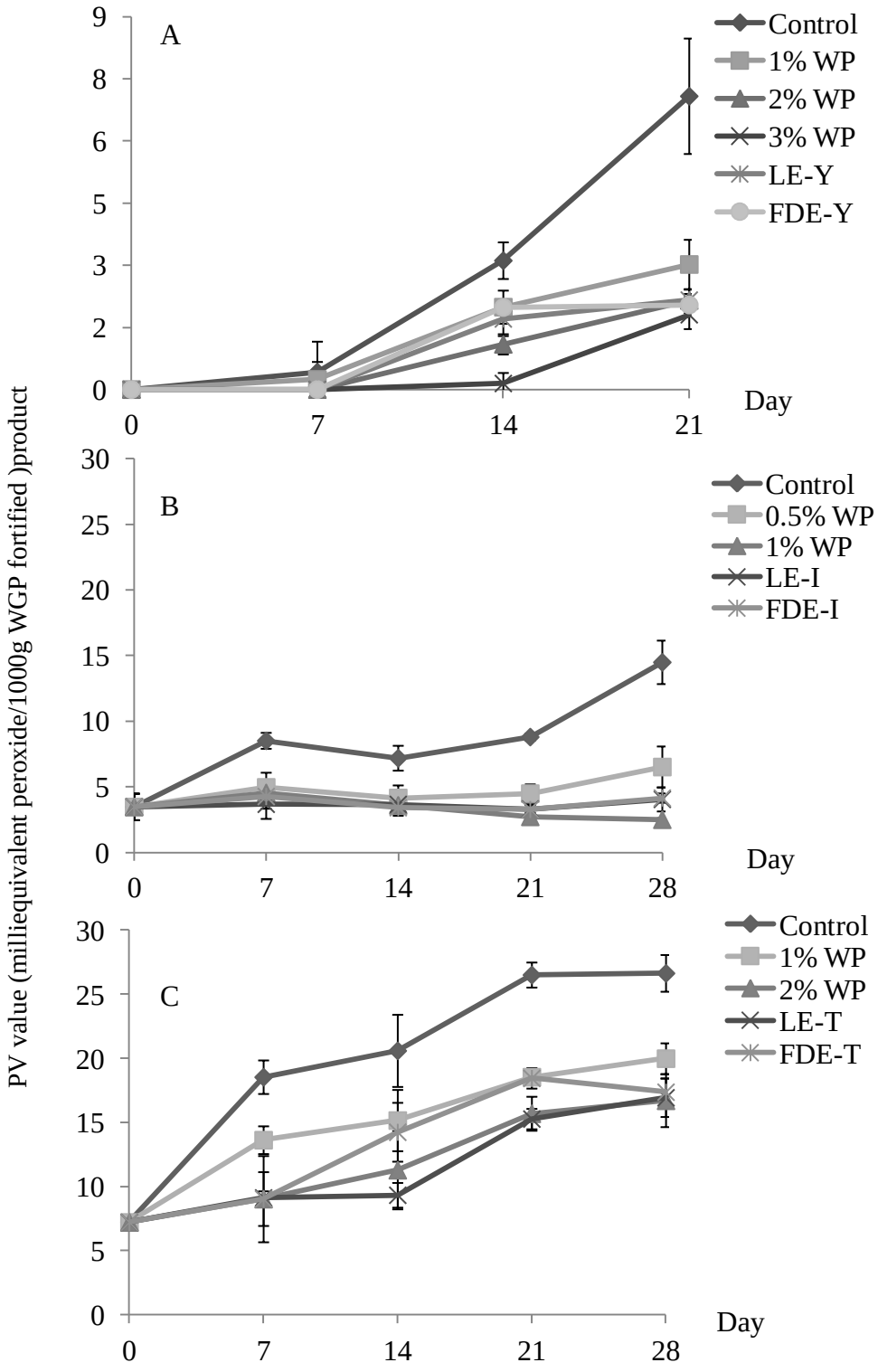

Fig. 2. Peroxide value of samples during storage at 4°C. (A) WGP fortified yogurt, (B) WGP fortified Italian salad dressing, and (C) WGP fortified Thousand Island salad dressing.

(Gamez-Meza et al., 2009); WGP fortified corn chips received lower peroxide value during storage (Rababah et al., 2011); flavanol oligomers from WGP were the most potent oxidation inhibitors for emulsions and frozen fish muscle (Medina, Pazos, Gallardo & Torres, 2005); and lipid stability in WGP added raw and cooked chicken was significantly increased (Sáyago-Ayerdi, Brenes & Goñi, 2009).

3.6 Dietary fiber fractions of WGP and WGP fortified yogurt and salad dressings

In WGP, IDF fraction took part of about 97-98% of TDF, while SDF fraction was only about 2% of TDF (Table 2). Those value were comparable with previous study (Llobera et al., 2007). The ratio of insoluble to soluble fraction, associated with the physiological effect, varied from 1.0 to 1.7 for fresh grape, whereas that of WGP was significantly higher, from 4.0 to 22.5 (González-Centeno, Rosselló, Simal, Garau, López & Femenia, 2010). In WGP fortified products, 3% WP (w/w yogurt) sample had the highest TDF of 3.2%, followed by 2% WP (w/w yogurt) one with about 1.9%. IDF contributed to the most of the fibers, in which 2% WP and 3% WP (w/w yogurt) samples had significantly ($P<0.05$) higher IDF, 3.1% and 1.9%, respectively. There was no significant difference ($P>0.05$) in SDF among all the samples, ranging from 0.04 to 0.07%. Although the 5% fiber-added commercial yogurt had 7.15% TDF, its TPC (855 mg GAE/kg, data not shown) was significantly less than that of 2% WP, 3% WP (w/w yogurt), LE-Y and FDE-Y fortified product. Also, no RSA was detected in commercial product (data not shown), indicated that WGP fortified yogurt had better antioxidant property.

For WGP fortified salad dressings, the highest TDF were detected in 0.5% WP (w/w Italian) and 1% WP (w/w Thousand Island) samples, 2.1% and 1.8%, respectively; whereas the least TDF were in FDE fortified samples, 0.8% and 1.0%, respectively. The higher TDF in WP added Italian sample was due to the sedimentary ingredients in the Italian salad dressing base calculated as klason lignin in IDF. Overall, WGP contributed significantly to the dietary fiber content in fortified products, especially the samples fortified with WP.

Dietary fibers from fruit and vegetable byproduct may be developed as food ingredients to offer the physiological functionalities on solubility, viscosity, hydration

property, oil-binding capacity and antioxidant activity on food products (Elleuch, Bedigian, Roiseux, Besbes, Blecker & Attia, 2011). Staffolo and others (2004) used apple wheat, bamboo and inulin as source of dietary fiber for improving rheological properties of yogurt. Sendra and others (2010) fortified yogurt with orange byproduct and showed increased viscosity and improved water absorption. Soukoulis and others (2009) reported that dietary fibers from oat, wheat, apple and inulin are able to control the crystallization and recrystallization in frozen dairy products by elevating the glass transition temperature.

Table 2.
Dietary fiber fractions of wine grape pomace (WGP) and WGP fortified yogurt and salad dressings *

		IDF	SDF	TDF
WGP		59.88 ± 1.64	1.44 ± 0.05	61.32 ± 1.69
WGP fortified Yogurt	1% WP	0.89 ± 0.00 c	0.04 ± 0.00 a	0.94 ± 0.01 c
	2% WP	1.92 ± 0.00 b	0.06 ± 0.00 a	1.98 ± 0.01 b
	3% WP	3.08 ± 0.01 a	0.07 ± 0.00 a	3.16 ± 0.01 a
	LE-Y	0.29 ± 0.00 c	0.05 ± 0.00 a	0.34 ± 0.00 c
	FDE-Y	0.74 ± 0.00 c	0.06 ± 0.00 a	0.80 ± 0.00 c
Commercial yogurt**		6.30 ± 1.18 a	0.86 ± 1.02 a	7.16 ± 2.20 a
WGP fortified Italian	0.5% WP	1.64 ± 0.02 b	0.09 ± 0.01 b	1.73 ± 0.02 b
	1% WP	2.00 ± 0.03 a	0.12 ± 0.01 a	2.12 ± 0.04 a
	LE-I	1.63 ± 0.04 b	0.06 ± 0.00 c	1.69 ± 0.04 b
	FDE-I	0.76 ± 0.01 c	0.05 ± 0.00 c	0.81 ± 0.02 c
WGP fortified Thousand Island	1% WP	1.50 ± 0.00 b	0.17 ± 0.02 b	1.66 ± 0.02 b
	2% WP	1.62 ± 0.01 a	0.21 ± 0.01 a	1.83 ± 0.02 a
	LE-T	1.32 ± 0.06 c	0.08 ± 0.00 d	1.40 ± 0.06 c
	FDE-T	0.88 ± 0.09 d	0.13 ± 0.00 c	1.02 ± 0.09 d

* Means followed by the same lowercase letters (a–d) in the same column within each concentration were not significantly different (P > 0.05). Control= no pomace added, WP= whole pomace powder, LE= pomace liquid extract, and FDE= freeze dried pomace extract.
** The commercial FiberOne yogurt contained 5% dietary fiber from blueberries (YoPlait, USA).

3.7 Total phenolic content (TPC) of WGP fortified yogurt and salad dressings

TPC of WGP fortified products increased along with increased WP concentration in the product, 732, 985 and 1338 mg GAE/kg yogurt for 1% WP, 2% WP and 3% WP (w/w yogurt), respectively. TPC in LE-Y and FDE-Y samples were higher than that in 2% WP (w/w yogurt), probably because the bioactive compounds in LE and FDE forms were easier to be extracted. Except 1% WP (w/w yogurt) sample, TPC content generally dropped during storage, with reduction rate of 39, 45 and 40% for 2% WP (w/w yogurt), LE-Y and FDE-Y samples, respectively. Similar trend was found by Karaaslan, Ozden, Vardin and Turkoglu (2011) that TPC in 10% Merlot grape extract fortified yogurt was 78 mg GAE/kg on the first day of storage, but decreased remarkably after 14 days of storage. Wallace and Giusti (2008) also reported that TPC degrades rapidly during the first week of storage, but is relatively stable after 2 weeks in yogurt fortified with berry and purple carrot extracts.

In WGP fortified Italian salad dressing, there was no difference ($P>0.05$) in TPC initially, ranged from 473 to 585 mg GAE/kg Italian salad dressing. Overall, TPC of all Italian dressing difference among 1% WP (w/w Italian), LE-I and FDE-I samples, in which FDE-I sample had samples decreased during storage. After 4 weeks of storage, there was no significant ($P>0.05$) the best retention with reduction rate of 16%., 2% WP (w/w Thousand Island) one had the highest TPC of 1339 mg GAE/kg dressing, and no significant decrease ($P>0.05$) in TPC during 4 weeks of storage.

Oxygen, pH, temperature, light, metal ions, enzymes and moisture content are the main factors influencing the retention of polyphenols (Mazza, 1995). Compared to the WGP fortified yogurt with pH of 4.4-4.6, salad dressing products with pH of 3.4-3.6 tended to have less reduction in TPC during storage, probably because the polyphenols were more stable under acidic condition. Friedman and Jürgens (2000) studied the effect of pH on the stability of phenolic compounds, and found that the susceptibility was different depending on the structure of the phenol, in which gallic acid and catechin, the major bioactive compounds in WGP, were unstable under high pH environment and irreversible during food process (Friedman et al., 2000). Gauche, Malagoli and Bordignon Luiz (2010) also indicated that pH 3.3 was the optimum for anthocyanin, the main bioactive compounds in WGP skin.

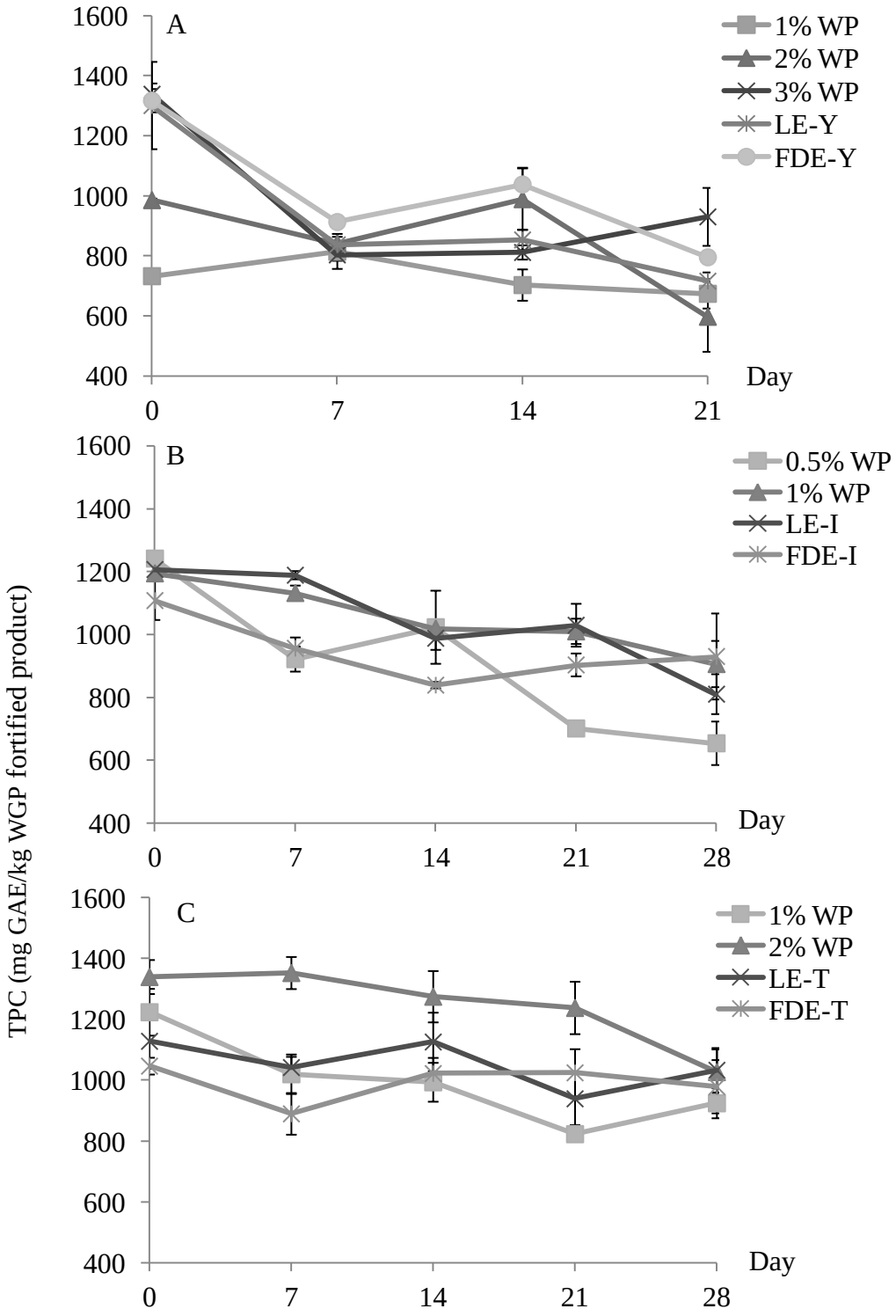

Fig. 3. Total phenolic content of samples during storage at 4°C. (A) WGP fortified yogurt, (B) WGP fortified Italian salad dressing, and (C) WGP fortified Thousand Island salad dressing.

In addition to the antioxidant activity, WGP has also shown good antimicrobial properties. The hydroxyl group in TPC could interact with the membrane protein of bacteria by hydrogen bonding and cause the changes in membrane permeability and cell destruction (Boulekbache-Makhlouf, Slimani & Madani, 2013; Puupponen-Pimiä et al., 2001). Özkan,Sagdiç, Göktürk Baydar and Kurumahmutoglu (2004) indicated that WGP could inhibit several spoilage and pathogenic bacteria and more effective against Gram-positive bacteria. In addition, resveratrol from grape pomace extract played an important role to prevent the fungal foodborne contamination in apple or orange juices (Sagdic, Ozturk, Ozkan, Yetim, Ekici & Yilmaz, 2011a).

3.8 Radical scavenging activity of WGP fortified yogurt and salad dressings

As expected, 3% WP (w/w yogurt) sample received the highest RSA of 936 mg AAE/kg yogurt initially, followed by 2% WP (w/w yogurt), LE-Y and FED-Y samples with RSA value of 603, 487 and 442 mg AAE/kg yogurt, respectively (Figure 4). RSA of 3% WP (w/w yogurt) significantly ($P<0.05$) dropped during storage, and was 645 mg AAE/kg yogurt at week 4, while the reduction rate was about 29, 52, 30 and 17% for 2% WP, 3% WP, LE-Y and FDE-Y samples, respectively. Karaaslan et al. (2011) stated that RSA declined 1.16 to 3.78 times in yogurt fortified with10% red grape extract after 14 days of storage.

In respect to salad dressings, RSA of WP fortified samples were significantly higher than those fortified with LE and FDE under same concentration, initially and during 4 weeks of storage (Figure 4). Initial RSA were 585 and 710 mg AAE/kg dressing for 1% WP (w/w Italian) and 2% WP (w/w Thousand Island), respectively. RSA dropped during storage with reduction rate of 30% and 18% for 1% WP (w/w Italian) and 2% WP (w/w Thousand Island) samples, respectively at the end of 4 weeks.

Oxygen accelerated the oxidation, leading the decline of RSA and increase of PV during storage. With the less RSA to remove the reactive oxygen species (ROS), those free radicals could initiate the lipid oxidation, thus increased PV. Hence, PV could serve as an indicator of the initial stage of oxidation and predict rancidity (Shahidi et al., 2005). TPC presents broader range of substrates on both free and bound phenolics in the products, while RSA provides more direct information on how capable to prevent ROS

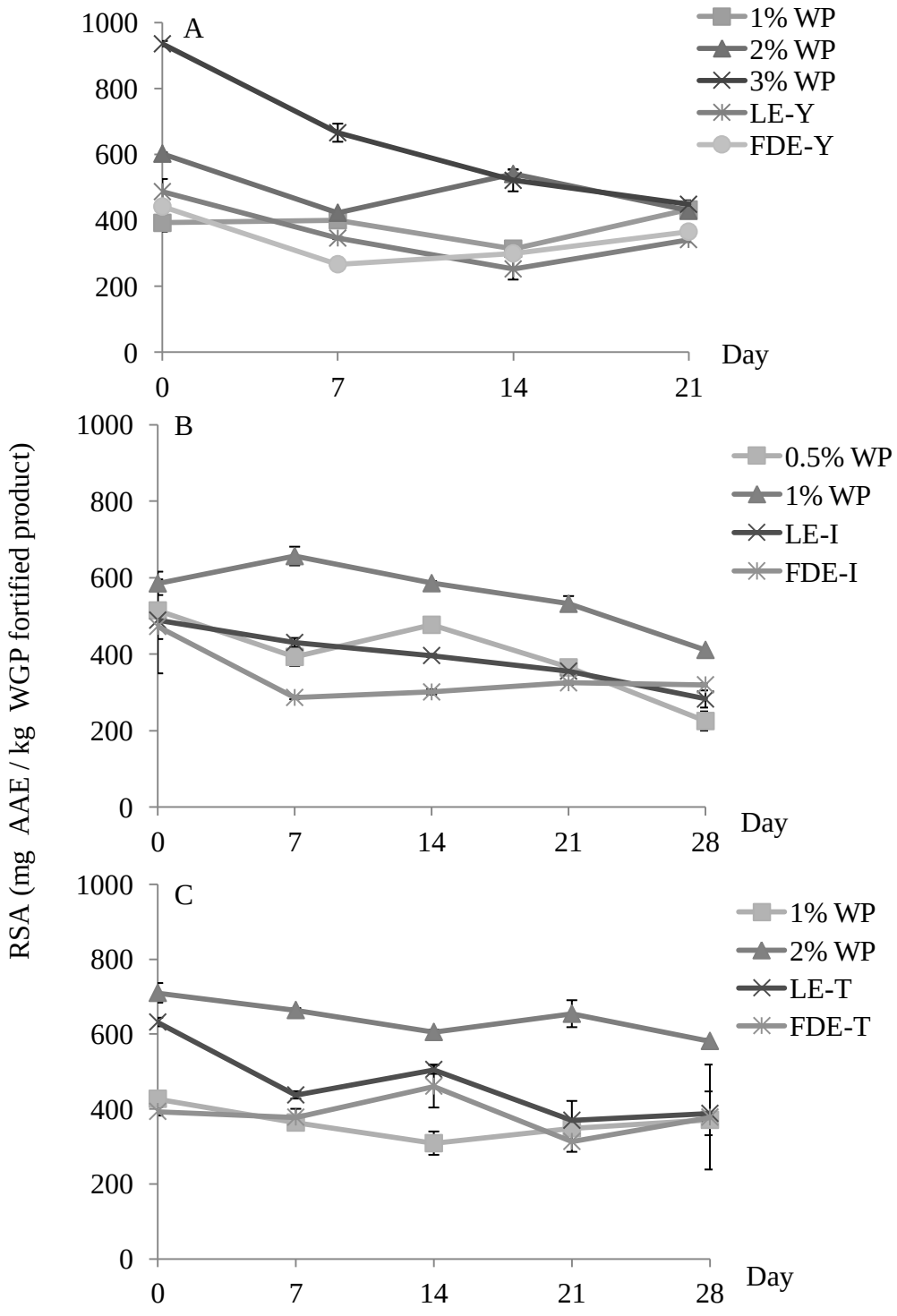

Fig. 4. DPPH radical scavenging activity of samples during storage at 4 °C. (A) WGP fortified yogurt, (B) WGP fortified Italian salad dressing, and (C) WGP fortified Thousand Island salad dressing.

from attacking lipoproteins, polyunsaturated fatty acids, DNA, amino acids and sugars in biological and food systems (Sagdic, Ozturk, Ozkan, Yetim, Ekici & Yilmaz, 2011b).

Another reason of the RSA drop in WGP fortified yogurt after first week of storage might be due to the protein-polyphenol interaction. The covalent binding between proteins and phenolic compounds released the free phenolic hydroxyl groups, which can act as antioxidants (Viljanen, Kylli, Kivikari & Heinonen, 2004). However, antioxidant activity from phenolic compounds can be masked by interactions with proteins (Heinonen, Rein, Satué-Gracia, Huang, German & Frankel, 1998). Arts et al. (2002) indicated that the masking depends on both type and amount of protein and bioactive compound, and the highest masking was observed in the combination of casein in milk with gallic acid in tea. In WGP fortified yogurt, casein in yogurt and gallic acid as a major phenolic compound in WGP acted masking effect, which might explain the significant TPC and RSA reduction in WGP fortified yogurt at the first week of storage.

3.9 Consumer acceptance of WGP fortified yogurt and salad dressings

In WGP fortified yogurt, appearance liking and overall liking among the control, 1% WP and 2% WP (w/w yogurt) samples were not scored differently ($P>0.05$) by the panelists (Table 5). However, 2% WP (w/w yogurt) sample received lower score on flavor and texture liking. Although equal numbers of panelists gave liking and disliking scores on the flavor of WGP fortified yogurt, more panelists ranked “like very much” on the flavor of 1% WP (w/w yogurt) than that of 2% WP (w/w yogurt) (data now shown). Also, 8 out of 12 panelists liked the texture of 1% WP (w/w yogurt), but only 3 out of 12 panelists liked the texture of 2% WP (w/w yogurt) (data not shown). The consistency scores showed that 1% WP (w/w yogurt) sample was the closest to “just about right”, neither too thick nor too thin. Some panelists indicated their appreciation on the nutritional value and fruity taste of WGP fortified yogurt, but others stated their disliking on the chalky and medical aftertaste which might come from the astringency of tannin in WGP.

In WGP fortified Italian dressing, overall, there was no difference ($P>0.05$) on all measured sensory attributes among control, 0.5% WP and 1% WP (w/w Italian) samples. In 0.5% WP (w/w Italian), 5, 5, 6 and 6 out of 12 panelists ranked “like very much” on

Table 5
Consumer acceptance of wine grape pomace (WGP) fortified yogurt and salad dressings *

		Appearance liking	Overall liking	Flavor linking	Texture linking	Consistency
WGP Fortified Yogurt	Control	6.58 ± 2.02 a	5.83 ± 2.29 a	6.25 ± 1.76 a	6.50 ± 1.68 a	2.50 ± 0.80 b
	1% WP	5.50 ± 2.07 a	4.83 ± 2.52 a	4.92 ± 1.98 b	5.83 ± 1.19 a	2.83 ± 0.58 a
	2% WP	5.83 ± 1.70 a	4.83 ± 1.95 a	4.75 ± 2.09 b	4.75 ± 1.54 b	2.75 ± 0.62 ab
WGP Fortified Italian	Control	5.83 ± 1.90 a	6.67 ± 1.15 a	6.75 ± 0.87 a	6.25 ± 0.97 a	2.58 ± 0.67 a
	0.5% WP	6.92 ± 1.24 a	7.08 ± 1.00 a	7.00 ± 1.28 a	6.83 ± 1.59 a	2.92 ± 0.90 a
	1% WP	6.50 ± 1.09 a	6.58 ± 0.90 a	6.42 ± 1.44 a	6.50 ± 1.38 a	2.83 ± 0.58 a
WGP Fortified Thousand Island	Control	6.85 ± 1.21 a	7.00 ± 1.22 a	6.69 ± 1.70 a	7.23 ± 1.30 a	3.31 ± 0.48 b
	1% WP	7.00 ± 0.82 a	6.62 ± 1.45 a	7.00 ± 1.29 a	6.85 ± 1.14 ab	3.46 ± 0.52 ab
	2% WP	6.08 ± 1.85 a	6.38 ± 1.80 a	6.46 ± 1.33 a	6.15 ± 1.34 b	3.92 ± 0.76 a

* Scale from 9 to 1. For liking attributes, 9 = like extremely and 1= dislike extremely; for consistency, 5=too thick, 1=too thin, and 3= just about right. Results are the mean of 12 replicates ± SD. Means followed by the same lowercase letters (a–d) in the same column within each concentration were not significantly different (P>0.05). Control= no pomace added and WP= dried whole pomace powder.

the appearance, overall, flavor and texture liking, respectively (data not shown). The consistency of 0.5% WP (w/w Italian) sample was also scored “just about right”. Most panelists commented that they like the healthy, less oily and taste of WGP fortified Italian dressing, but a few panelists pointed that the fortified one is too sour.

In respective to WGP fortified Thousand Island dressing, there was no significant difference ($P>0.05$) on appearance, overall and flavor liking among control, 1% WP and 2% WP (w/w Thousand Island) samples. Over10 panelists ranked liking on 1% WP (w/w Thousand Island) sample on appearance, overall, flavor and texture, while over 7 panelists ranked liking on 2% WP (w/w Thousand Island) (data not shown). The 2% WP (w/w Thousand Island) sample was thicker in the texture, which might make some panelists disliking the product. In summary, WGP fortified yogurt and salad dressing were well accepted by consumer, but the amount of WP added into the products was less based on consumer sensory study than that from the analytical results.

4. Conclusion

This study demonstrated that Pinot Noir wine grape pomace may be utilized as an alternative source of antioxidant dietary fiber to fortify yogurt and salad dressing for not only increasing dietary fiber and total phenolic content, but also delaying lipid oxidation of samples during refrigeration storage. Although products fortified with the pomace extracts (liquid and freeze dried) obtained the most similar physicochemical properties to the control (no pomace added), those fortified with dried whole pomace powders (WP) had higher dietary fiber content. Unfortunately, total phenolic content (TPC) and DPPH radical scavenging activity (RSA) of fortified samples decreased during storage, in which more reduction was observed in yogurt than that in salad dressings, probably due to the interactions between proteins in yogurt and phenolic compounds in pomace. Therefore, it is necessary to further investigate the mechanisms and methods of retention of TPC and RSA in the products in the future studies by using chromatographic techniques to profile the change of pheonolic compounds. Based on the balance in DF and TPC contents, RSA value, physicochemical qualities and consumer acceptance, the best received products were 1% (w/w) WP fortified yogurt, 0.5% (w/w) WP fortified Italian dressing, and 1% (w/w) WP fortified Thousand Island dressing.

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CHAPTER 5. CONCLUSION

This thesis research developed the feasible drying methods for wine grape pomace (WGP) preparation for retaining the pheonlic compounds, antioxidant and antimicrobial activities under long-term storage and demonstrated the feasibility of fortifying WGP in yogurt and salad dressing.

The results confirmed that Pinot Noir WGP, containing seeds and skins or skins only, met the definition of ADF when subjected to the four different dehydrate methods initially. Although freeze dry retained higher amount of TPC than ambient air dry, 40 ^{o}C oven and vacuum dry initially, the difference with other measured bioactive compounds and antioxidant activity were not significant. After 16 weeks of storage at 15 °C, WGP containing both seeds and skins still met the criteria of ADF. Based on the antibacterial activity study, minimum inhibition concentration was negatively correlated to total phenolic content and WGP was more effective against Gram-positive bacteria. Therefore, 40 °C oven and ambient air dry are highly acceptable by considering the amount of retention of the measured bioactive compounds and antioxidant activity due to their much less cost compared with freeze dry. WGP may be prepared in commercial scale by applying these economically feasible drying methods for late applications.

This study also demonstrated that WGP as ADF functional food ingredient can be fortified in yogurt and salad dressing. Based on the balance in dietary fiber, total phenolic contents, DPPH radical scavenge activities, physicochemical qualities and consumer acceptances, the best received products were 1% (w/w) WGP whole powder (WP) fortified yogurt, 0.5% (w/w) WP fortified Italian dressing, and 1% (w/w) WP fortified Thousand Island dressing. Although products incorporated with the pomace extracts (liquid and freeze dried) obtained the most similar physicochemical properties to the control (no pomace added), those products fortified with dried WP had higher dietary fiber content due to the insoluble fractions. Thus, WGP fortified products not only extended the shelf life by controlling lipid oxidation of products during storage, but also increased the nutrition value for enhancing the phenolic compounds and dietary fiber contents.

Several suggestions are worth to investigate for the future studies. First, it is necessary to profile the pheonlic by HPLC to determine the change of individual compounds during storage time. Secondly, WGP may be fortified in other high lipid foods to evaluate its feasibility of applications. Last, the *in vivo* study in animal and human subjects should be applied to future evaluate its health promotion benefits when fortified in food products.