



## Wine grape pomace as antioxidant dietary fibre 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 fibre (ADF) was fortified in yogurt (Y), Italian (I) and Thousand Island (T) salad dressings. During the 3 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 fibre 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 was 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.

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### 1. Introduction

The concept of antioxidant dietary fibre (ADF) was first proposed by Saura-Calixto (1998) with the criteria that 1 g of ADF should have DPPH free radical scavenging capacity equivalent to at least 50 mg vitamin E and dietary fibre content higher than 50% dry matter from the natural constituents of the material. Wine grape pomace (WGP), the residual seed and skins from winemaking, contains high phenolic compounds and dietary fibre (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 fibres from grapes show higher reducing efficacy in lipid profile and blood pressure than that from oat fibre or psyllium due to combined effect of dietary fibre 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 fibre, and most of the beneficial bioactive compounds

remained in those byproducts (Balasundram, Sundram, & Samman, 2006). ADF may be incorporated with flour for making high dietary fibre bakery goods, while the polyphenols in ADF could contribute as antioxidant for improving colour, 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 fibre 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 fibres. Fruit are commonly blended in after milk is fermented to make stirred yogurt that is

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non-Newtonian with weak viscoelastic property (Lubbers, Decourcelle, Vallet, & Guichard, 2004). The effects of different types of fruit as source of dietary fibre on the rheological properties of yogurt have been studied (Sendra et al., 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 et al., 2008), ascorbyl palmitate,  $\alpha$ -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 fibre 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 fibre content was 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 °C. Yogurt was further analysed 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  $10,000 \times g$  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 was 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 & Zhao, 2012). Dietary fibre (DF), including soluble (SDF) and insoluble dietary fibre (IDF) fractions, was analysed 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 °C for 30 min and then centrifuged. IDF was obtained from the residues, while SDF was the supernatant.

SDF fraction was dialysed in deionized water by the tubing with a molecular weight cutoff of 12,000–14,000 (Spectrum Laboratories, Inc., USA) for 48 h. The dialysate was freeze-dried and hydrolysed with 72% sulphuric acid at 121 °C 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<sub>2</sub>SO<sub>4</sub> and boric acid–sodium chloride solution was incubated at 70 °C for 40 min, the solvent was then treated with 3,5-dimethylphenol–glacial acetic acid (Sigma Chemical Co., USA) and the absorbance was measured at 400 and 450 nm, respectively. SDF was calculated by the sum of NS and UA.

IDF fraction was hydrolysed by 72% sulphuric acid at 30 °C for 1 h, followed at 121 °C 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 °C. IDF was quantified by the sum of KL, NS and UA, and total dietary fibre 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<sub>3</sub> 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-picrylhydrazyl (DPPH) (Kasel Kogyo Co. Ltd., Japan) assay based on ascorbic acid (Mal-linckrodt 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 equivalent (AAE)/g WGP.

### 2.4. Preparation of yogurt and salad dressing

Yogurt was prepared using reduced fat milk (2% milk fat, Daringold, USA) with 4% sugar (w/v milk) addition. Sugar was dissolved in the milk and pasteurized in 85 °C water bath for 30 min and

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