JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

# Concord Grape Pomace Polyphenols Complexed to Soy Protein Isolate Are Stable and Hypoglycemic in Diabetic Mice

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**ABSTRACT:** Polyphenols extracted from Concord grape pomace were stabilized by complexation to soy protein isolate (SPI) to produce grape polyphenol−SPI complex (GP−SPI) containing 5% or 10% grape polyphenols. LC−MS and MALDI-TOF analysis showed that a broad range of phytochemicals were present in the grape pomace extract. Anthocyanins and total polyphenols in the GP−SPI complex were stable after a 16-week incubation at 37 °C but were reduced by up 60% in dried grape pomace extract. Compared to vehicle (236 ± 34 mg/dL), a single dose of 300 mg/kg GP−SPI (184 ± 32 mg/dL) or 500 mg/kg GP−SPI (177 ± 28 mg/dL) having 5% grape polyphenols significantly lowered blood glucose in obese and hyperglycemic C57BL/6 mice 6 h after administration. GP−SPI allows the capture of grape pomace polyphenols in a protein-rich food matrix and may be useful as a functional food ingredient for the management of blood glucose levels.

**KEYWORDS:** *grape pomace, polyphenols, soy protein isolate, nutrition, hyperglycemia*

## INTRODUCTION

Grapes are consumed worldwide in the form of fresh or dried fruit, preserves, juice, or wine. Anthocyanins, tartarate esters of hydroxycinnamic acids, and proanthocyanidins represent 85% of the phytochemical compounds present in grapes, while the other 15% comprise monomeric flavan-3-ols, flavonols, hydroxybenzoic acids, free hydroxycinnamic acids, and stilbenes such as resveratrol.[1] The antioxidant polyphenols in grapes have been associated with antiaging and antineurodegenerative effects,[2] reduction in atherosclerosis, and improved endothelial function.[3] Grape polyphenols have also been reported to reduce obesity-induced chronic inflammation by multiple mechanisms, which may aid in prevention of metabolic disease such as metabolic syndrome and type 2 diabetes (T2D).[4]

Polyphenols have a natural affinity for proteins and generally form complexes with flexible proline-rich proteins that possess an open conformation.[5,6] Polyphenol−protein interactions are dependent on pH, temperature, the three-dimensional distribution of amino acid residues on protein surfaces or cavities, and the space available within the protein cavity to accommodate variably sized polyphenol molecules.[7,8] We have previously demonstrated that protein-rich, plant-derived foods, such as defatted soybean flour (DSF), can sorb, concentrate, and stabilize polyphenols from Concord grape (*Vitis labrusca*) juice[9] as well as from other fruit juices[10,11] while leaving behind most water and sugars. Grape polyphenol−enriched DSF showed hypoglycemic activity in obese and hyperglycemic C57BL/6 mice, indicating that the DSF matrix can effectively capture and preserve bioactive polyphenols from grape juice.[9] Grape pomace, a byproduct of the juice and wine industries, is composed mainly of grape seeds and skins. Although high in bioactive polyphenols such as anthocyanins, catechins, proanthocyanidins, and flavonol glycosides,[12] grape pomace is not a palatable source of dietary polyphenols and is usually discarded.

Soy protein isolate (SPI) prepared from soybeans (*Glycine max*) is an inexpensive protein frequently incorporated into foods and supplements. In comparison to casein, SPI was found to be hypocholesterolemic,[13] to decrease activity of lipogenic enzymes,[14] and to reduce body fat in obese rats and mice.[15] In this study we have optimized a food-compatible method to extract polyphenols from Concord grape pomace and complex them to SPI. The resulting grape polyphenol−SPI complex (GP−SPI) was investigated for long-term stability at 37 °C and acute hypoglycemic activity in the C57BL/6 diabetic mouse model. GP−SPI offers a strategy to capture, stabilize, and effectively deliver valuable phytochemicals from the byproducts of juice and wine manufacturing.

**Received:** July 23, 2013
**Revised:** October 30, 2013
**Accepted:** November 8, 2013
**Published:** November 8, 2013





dx.doi.org/10.1021/jf403238e | *J. Agric. Food Chem.* 2013, 61, 11428−11433

## MATERIALS AND METHODS

**Materials and Chemicals.** Experiments were performed using frozen depectinized Concord grape pomace obtained from Milne Fruit (Prosser, WA) or Welch's (Concord, MA). Soy protein isolate (SPI) was donated by Archer Daniels Midland (Decatur, IL). Folin−Ciocalteu reagent, proanthocyanidin B2, and gallic acid were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). HPLC grade organic solvents were obtained from VWR International (Suwanee, GA, USA).

**Quantification of Total Monomeric Anthocyanins, Total Proanthocyanidins, and Total Polyphenols.** The pH differential method[16] was used to measure total monomeric anthocyanins in Concord grape pomace extracts. Absorbance was measured at 520 and 700 nm with a UV/vis spectrophotometer (Shimadzu UV-2450 or Synergy HT Multi-Detection Microplate Reader, BioTek), and the concentration of total monomeric anthocyanins (mg/L) was calculated as cyanidin 3-*O*-glucoside equivalents. Proanthocyanidins (mg/L) were quantified using the DMAC method and reading samples at 640 nm using proanthocyanidin B2 as standard.[17] Total polyphenols (mg/L) were quantified by the Folin−Ciocalteu method,[18] and samples were read at 726 nm against a gallic acid standard curve.

**Optimization of Method for Concord Grape Pomace Extraction.** Twenty grams (wet weight) of Concord grape pomace was pureed in a Vitamix blender with 200 mL of 50% ethanol (1:10 extraction ratio). The puree was transferred to a rotary evaporation flask, attached to a rotary evaporator (Büchi, Switzerland), and extracted at 80 °C with constant rotation (105 rpm) and ambient pressure. An aliquot of the pureed sample was removed before incubation at 80 °C and then after 1, 2, and 3 h of extraction to determine optimal extraction time. Samples were allowed to cool to room temperature and then centrifuged at 4000 rpm for 10 min (Eppendorf, model 5810R) to separate the solids from the extract. The concentration of total polyphenols (mg/L) was quantified using the Folin−Ciocalteu method as described.[18]

To determine optimal pH for extraction, depectinized grape pomace (30 g wet weight) was pureed with 300 mL of 50% ethanol, the puree was aliquoted into 40 mL volumes in 50 mL tubes, and pH was adjusted from 4.3 (native pH) to 2, 3, or 5 with HCl or NaOH. The material was then extracted in loosely capped tubes in an 80 °C water bath for 2 h. The samples were centrifuged at 4000 rpm for 10 min (Eppendorf, model 5810R) to separate the solids from the liquid extract. The concentrations of total polyphenols and proanthocyanidins were quantified as described above.

To determine the optimal solids to solvent extraction ratio, triplicate samples of grape pomace were pureed using 50% ethanol at a 1:10 or 1:5 extraction ratio (wt/vol). For the 1:10 extraction 20 g of pomace and 200 mL of 50% ethanol were pureed in the Vitamix blender, and for the 1:5 extraction 20 g of pomace and 100 mL of 50% ethanol were pureed. The pH of each mixture was adjusted to 2.0 using sulfuric acid ($H_2SO_4$). The mixtures were transferred to flasks and extracted in an 80 °C water bath for 2 h under constant rotation in a rotary evaporator without vacuum (Büchi, Switzerland). The samples were centrifuged at 4000 rpm for 10 min to separate the solids from the extract. The volumes of recovered extract were measured. The concentrations of total polyphenols, proanthocyanidins, and anthocyanins were quantified in extracts as described above. Aliquots of each extract (100 $\mu$L) were lyophilized to determine the amount of solids in liquid extract (mg/mL). This information was used to calculate the percentage of anthocyanins, proanthocyanidins, and total polyphenols in dried grape pomace extract.

**Biochemical Characterization of Grape Pomace Extract.** Concord grape pomace was pureed together with 50% ethanol (1:5 solids/solvent ratio). $H_2SO_4$ was added to adjust the pH of the mixture to 2, and extraction was performed for 2 h at 80 °C with agitation. Grape pomace extract (3 mL) was diluted five times with doubly distilled water ($ddH_2O$). The total volume of the diluted extract was applied to a c18 Sep-Pak that had been conditioned with methanol and equilibrated with $ddH_2O$. The Sep-Pak was sequentially rinsed with (1) 50 mL of $ddH_2O$ to remove nonphenolic compounds, (2) 50 mL of 10% aqueous methanol (v/v) to remove low molecular weight polyphenols, and (3) methanol (0.1% trifluoroacetic acid) to remove proanthocyanidins. The methanol eluate was evaporated to dryness under a stream of nitrogen and reconstituted in ethanol for MALDI-TOF MS analysis.

Mass spectra were collected on a Bruker ULTRAFLEX III MALDI TOF/TOF mass spectrometer (Billerica, MA, USA) equipped with delayed extraction and a SmartBeam laser. All analyses were performed in a positive reflectron mode. Spectra were the sum of 8−10 different locations in each well, accumulating a total of 400−500 shots to minimize intrawell patterns. The matrix was 2,5-dihydroxybenzoic acid (DHB) at a concentration of 50 mg/mL in ethanol. FlexControl and FlexAnalysis (Bruker Daltonik GmbH, Bremen, Germany, version 3.0) were used for data acquisition and data processing, respectively. mMass (version 3.9.0) was used for spectra analysis. On the basis of previously described structures, an equation was developed to predict the mass distribution of polygalloyl polyflavan-3-ols (PGPFs) in grape products. The equation is $290 + 288c + 152g + 23$, where 290 represents the molecular weight of the terminal catechin/epicatechin unit, $c$ is the degree of polymerization of catechin/epicatechin units, $g$ is the number of galloyl esters, and 23 is the molecular weight of sodium. MALDI-TOF MS analysis of grape pomace extract shows a series of masses that correspond to the PGPFs that have been previously described in grape products (Krueger et al.[21]).

**Pilot Scale Production of GP−SPI Complex.** After determination of the optimal conditions for extraction of depectinized Concord grape pomace, the process was applied to extraction of a pilot scale batch of pomace in a commercial manufacturing facility (Cyalume Technologies, Bound Brook, NJ). Grape pomace (150 kg) was mixed with 750 L of 50% ethanol (1:5 solids/solvent, wt/vol ratio), and $H_2SO_4$ was used to adjust the pH of the mixture to 2. Extraction of pomace was performed at 80 °C for 2 h with agitation and ambient pressure. The mixture was allowed to cool, and the solids and pomace extract were separated by filtration. The concentrations of total polyphenols and anthocyanins were quantified in the extract as described in methods. The extract dry weight was determined by drying triplicate 1 mL samples of the extract in a speed vacuum. The percentage of total polyphenols per dry weight was determined; the difference between the dry weight of the pomace extract and the dry weight of total polyphenols represents nonpolyphenolic components. A calculated amount of soy protein isolate (SPI; ADM, Decatur, IL) was added to the liquid grape pomace extract and mixed for 30 min so that after evaporation of the solvent a grape pomace polyphenol−SPI complex (GP−SPI) containing 10% total polyphenols was produced. Example calculation is as follows: 100 g of TP + (150 g of total dried extract − 100 g of TP) + (X g of SPI) = 1000 g; X = 850 g of SPI. The pomace extract−SPI mixture was dried to 5% moisture content (first in the mixing vessel and then in a tray drier) at 50 °C under vacuum. The yield was 15 kg of GP−SPI, which was milled to a powder.

**Stability of Polyphenols in the GP−SPI Complex Compared to Dried Grape Pomace Extract.** An aliquot (80 mL) of the grape pomace extract was freeze-dried. The stability of dried grape pomace extract and GP−SPI (10% total polyphenols) was compared by incubating both powders in closed 50 mL tubes at 37 °C. Before incubation and at various times after, 1 g aliquots of both powders were removed. The GP−SPI was extracted three times with 50% acetone at a 1:10 ratio (solid/solvent, wt/vol), vortexing for 30 s and centrifuging at 4000 rpm for 5 min to collect the supernatants, which were pooled. The grape pomace extract was resuspended in 50% acetone, vortexed for 30 s, and centrifuged to remove insoluble particles. Total polyphenols and anthocyanins were quantified in the extracts as described in methods.

**Acute Hypoglycemic Effect of GP−SPI Complex High Fat Diet-Fed C57BL/6J Mice.** GP−SPI was prepared as described in methods except a calculated amount of SPI was added to the grape pomace extract so that the GP−SPI contained 5% total polyphenols. The following protocol was approved by Rutgers University Institutional Care and Use Committee and followed federal and state laws. Five-week-old male C57BL6/J mice (10−20 g) were purchased from Jackson Laboratory (Bar Harbor, ME) and fed a regular diet ad libitum (Purina, no.5015) during a 1 week

acclimatization period. At 6 weeks of age, mice were placed on a very high-fat diet (VHFD, 60% kilocalories fat, Research Diets D12492) for 12 weeks, which led to obesity, insulin resistance, and hyperglycemia. Body weights were measured weekly. Mice were randomly divided into experimental groups, fasted for 4 h, and then gavaged with the indicated doses of GP−SPI or SPI control (300 mg/kg) formulated in a 75% Labrasol−water solution (vehicle). Animals were fasted during the test period, and blood glucose readings were taken using a glucometer (AlphaTRAK 32004-02, Abbott Animal Health). Metformin (300 mg/kg) was administered as positive control.

**Statistical Analysis.** Statistics were performed with STATISTICA, version 10 (StatSoft). One-way ANOVA was used to determine significance among three or more groups followed by the indicated post hoc test. Paired *t* tests were performed within groups (before vs after treatment), and unpaired *t* tests were used for comparison of independent groups.

## RESULTS AND DISCUSSION

**Optimization of Concord Grape Pomace Extraction.** Optimal conditions for the extraction of frozen Concord grape pomace with 50% ethanol were determined with respect to extraction time, pH, and solids/solvent ratios. To determine optimal extraction time, grape pomace was blended with 50% ethanol (10:1 extraction) and extracted for 0, 1, 2, or 3 h at 80 °C with agitation. The concentration and amount of total polyphenols extracted increased with increased extraction time (Table 1). The 3 h incubation only gave a 0.1% increase in yield of total polyphenols compared to the 2 h incubation; therefore, the latter was selected for subsequent experiments. To evaluate extraction efficiency at different pH, grape pomace was blended in 50% ethanol (10:1 extraction) and the pH of 40 mL aliquots was changed from native pH 4.3 to pH 2, 3, or 5 prior to extraction with rotation at 80 °C for 2 h. Extraction of the pomace at pH 2 resulted in the highest concentrations of total polyphenols and proanthocyanidins (Table 2). To determine the optimal extraction ratio, pomace was blended with 50% ethanol in 1:10 or 1:5 (solids:solvent) ratios, slurries were adjusted to pH 2 with $H_2SO_4$, and samples were incubated at 80 °C for 2 h with agitation. The concentration and amount of total polyphenols, proanthocyanidins, and anthocyanins extracted were compared for the 1:5 and 1:10 solids/solvent ratio (Table 3). The concentration and amount of total polyphenols extracted using a 1:5 extraction ratio were significantly higher (two-tailed *t* test, $p < 0.01$) compared to the 1:10 extraction ratio (Table 3). There was no significant difference in concentration of proanthocyanidins, but the amount of proanthocyanidins extracted was significantly higher (two-tailed *t* test, $p < 0.01$) using the 1:10 solids/solvent ratio. The concentration and amount of anthocyanins did not differ significantly between the 1:5 and 1:10 extractions (Table 3). On the basis of these data, the optimal conditions for extracting total polyphenols from grape pomace were a 1:5 (solids/solvent) ratio of grape pomace in 50% ethanol adjusted to pH 2 incubated at 80 °C for 2 h with agitation.

**Table 1. Incubation Time for Extraction of Concord Grape Pomace[a]**

| time (h) | TP (mg/L) | TP (mg) | TP yield (%) |
|---|---|---|---|
| 0 | 458 | 73.3 | 1.0 |
| 1 | 1087 | 173.9 | 2.4 |
| 2 | 1284 | 205.4 | 2.9 |
| 3 | 1358 | 217.3 | 3.0 |

[a]TP = total polyphenols in gallic acid equivalents. Pomace (20 g) was extracted in 200 mL of 50% ethanol at 80 °C at unadjusted pH 4; 160 mL of extract was recovered. % TP yield is based on pomace dry weight of 36% (7.2 g per 20 g of wet weight).

**Table 2. Extraction of Concord Grape Pomace at Different pH Values[a]**

| pH | TP (mg/L) | PAC (mg/L) |
|---|---|---|
| 2 | 2000 | 1449 |
| 3 | 1602 | 1058 |
| 4.2 (native) | 1191 | 874 |
| 5 | 889 | 631 |

[a]TP = total polyphenols in gallic acid equivalents; PACs = proanthocyanidins in proanthocyanidin B2 equivalents. Pomace (30 g) was extracted in 300 mL of 50% ethanol at 80 °C at the indicated pH.

**Biochemical Characterization of Concord Grape Pomace Extract.** Concord grapes contain a large collection of anthocyanins as well as flavonols, hydroxycinnamic acids, catechins and proanthocyanidins.[9] Frozen Concord grape pomace was extracted using the optimized conditions described above, and the extract was filtered prior to compound characterization by LC−MS and MALDI-TOF MS. Table 4 summarizes putatively identified compounds from grape pomace extract. According to relative peak mass area, anthocyanins were the most abundant compounds detected, followed by catechins, hydroxycinnamic acids, and flavonols. MALDI-TOF MS is well-suited for characterizing polydispersed oligomers such as proanthocyanidins, which exhibit large structural heterogeneity.[19] The method produces a singly charged molecular ion for each parent molecule, allowing precise detection of high mass[20] and baseline detection of a broad range of proanthocyanidin oligomers over the degree of polymerization (DP) range of 2−23.[19] MALDI-TOF MS analysis of the grape pomace extract confirmed a series of masses corresponding to a proanthocyanidin series $[M + Na]^+$ from 3 to 13 DP ($m/z$ 889 to $m/z$ 3771), which have previously been described in grape products (Figure 1).[21] The data indicate that the described method for the extraction of Concord grape pomace efficiently releases a broad range of phenolic and polyphenolic compounds.

**Production and Stability of GP−SPI.** Polyphenol−protein complexes are known to form through hydrophobic and hydrogen bond interactions.[7] Leveraging this natural affinity between proteins and polyphenols, we mixed in a calculated amount of soy protein isolate (SPI) to the polyphenol-rich Concord grape pomace extract. The solvent was evaporated and the mixture was dried to a powder to create a novel food ingredient made of stable, three-dimensional, Concord grape polyphenol−SPI complex (GP−SPI) containing 10% total polyphenols. Ethanol has been reported to suppress the interaction between bovine albumin (BSA) protein and nonpolar tannins but not the interaction between BSA and more polar tannins.[22] The described process uses 50% ethanol to extract grape pomace; however, polyphenol−protein complexes should form without disruption as the solvent is gradually removed during production of GP−SPI, allowing uniform sorption of polyphenols onto surfaces of protein particles. In addition to lab-scale quantities, a pilot scale batch of GP−SPI (15 kg) containing 10% total polyphenols was produced in a commercial manufacturing facility, demonstrating that production of the GP−SPI matrix is readily scalable

**Table 3. Extraction of Concord Grape Pomace at Different Solid/Solvent Ratios[a]**

| solids/solvent ratio | vol (mL) | TP (mg/L) | TP (mg) | PACs (mg/L) | PACs (mg) | ACNs (mg/L) | ACNs (mg) |
|---|---|---|---|---|---|---|---|
| 1:5 | 71 ± 4.4 | 2417 ± 167** | 171.8 ± 18.4** | 1883 ± 340 | 132.7 ± 15.2 | 87 ± 16 | 6.1 ± 0.7 |
| 1:10 | 157 ± 7.0 | 1556 ± 160 | 94.5 ± 19.4 | 1398 ± 219 | 218.7 ± 27.8** | 62 ± 17 | 9.7 ± 1.8 |

[a]TP = total polyphenols in gallic acid equivalents. PACs = proanthocyanidins in proanthocyanidin B2 equivalents. ACNs = total monomeric anthocyanins. Two tailed *T*-test: (**) $p < 0.01$.

**Table 4. Compounds Extracted from Concord Grape Pomace**

| | | ESI MS ($m/z$) | | |
|---|---|---|---|---|
| compd | peak mass area ($\times 10^6$) | $[M]^+$ | $[M - H]^+$ | $t_R$ (min) |
| cyanidin-3-*O*-glucoside | 18.0 | 449 | | 4.73 |
| delphinidin-3-*O*-glucoside | 10.0 | 465 | | 4.16 |
| malvidin-3-*O*-glucoside | 5.86 | 493 | | 6.48, 6.60 |
| peonidin-3-*O*-glucoside | 4.70 | 463 | | 6.40 |
| petunidin-3-*O*-glucoside | 9.77 | 479 | | 4.94, 5.13 |
| cyanidin-3-*O*-(6″-*O*-acetyl)glucoside | 2.64 | 491 | | 11.97, 12.51 |
| dephinidin-3-*O*-(6″-*O*-acetyl)glucoside | 3.65 | 507 | | 8.56, 9.0 |
| cyanidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside | 14.7 | 595 | | 24.6 |
| delphinidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside | 17.5 | 611 | | 20.6 |
| malvidin-3-*O*-(6″-*O*-*p*-coumaroyl)glucoside | 20.01 | 639 | | 12.2 |
| peonidin-3-*O*-(6″-O-*p*-coumaroyl)-5-*O*-diglucoside | 5.965 | 771 | | 11.5 |
| delphinidin-3-*O*-(6″-*O*-*p*-coumaroyl)-5-*O*-diglucoside | 21.48 | 773 | | 10.1 |
| petunidin-3-*O*-(6″-*O*-*p*-coumaroyl)-5-*O*-diglucoside | 9.395 | 787 | | 10.8 |
| malvidin-3-*O*-(6″-*p*-coumaroyl)-5-*O*-diglucoside | 15.88 | 801 | | 11.5 |
| quercetin | 0.73 | | 301 | 23.72 |
| quercetin-3-*O*-glucoside and -galactoside | 0.561 | | 463 | 13.6 |
| quercetin-3-*O*-glucoronide | 2.07 | | 477 | 12.8 |
| myricetin-*O*-glycoside | 0.584 | | 479 | 8.99 |
| myricetin-*O*-glucoronide | 0.216 | | 493 | 8.26, 8.12 |
| coumaric acid | 2.79 | | 163 | 7.44, 7.70 |
| caffeic acid | 0.682 | | 179 | 5.49 |
| coutaric acid | 4.54 | | 295 | 4.76 |
| caftaric acid | 4.35 | | 311 | 4.14 |
| fertaric acid | 1.82 | | 325 | 5.24 |
| (epi)C−(epi)C dimer | 0.359 | | 577 | 11.89 |
| catechin | 3.204 | | 289 | 9.3 |
| (epi-)catechin | 11.45 | | 289 | 4.71, 5.30 |
| epicatechin 3-*O*-gallate | 3.91 | | 441 | 10.42 |

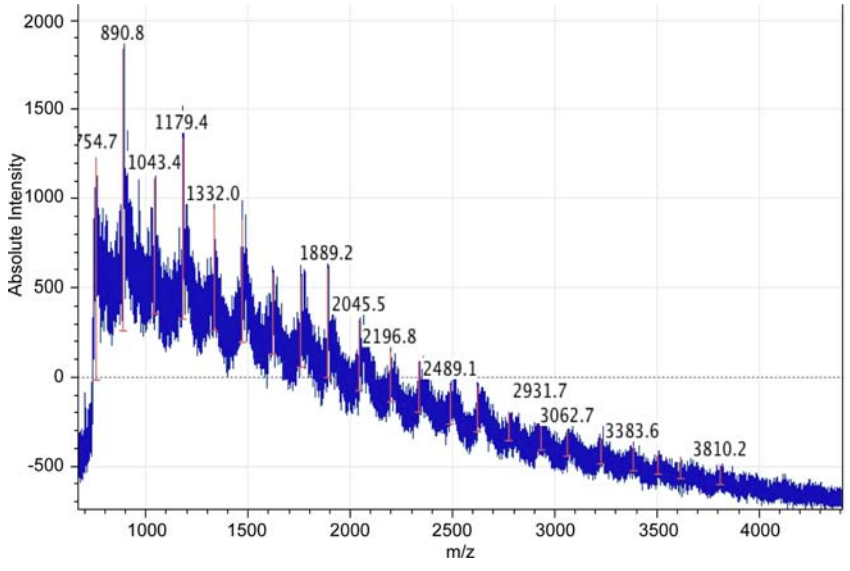


**Figure 1.** MALDI-TOF mass spectrum of Concord grape pomace extract. Data were generated in positive linear mode and shows a proanthocyanidin series $[M + Na]^+$ from 3 degrees of polymerization (DP, $m/z$ 889) to DP 13 ($m/z$ 3771).


dx.doi.org/10.1021/jf403238e | *J. Agric. Food Chem.* 2013, 61, 11428−11433

and compatible with manufacturing equipment commonly used in food production facilities.

To determine if complexation to SPI improves stability of grape polyphenols, the GP−SPI powder and dried grape pomace extract powder were incubated at 37 °C. The stability of anthocyanins and total polyphenols was evaluated over the course of 16 weeks as described in methods and expressed as a percentage of the original amounts that were eluted on day 0. The levels of anthocyanins and total polyphenols in the GP−SPI powder remained remarkably stable during the 16-week incubation period at 37 °C (Figure 2). In contrast, the levels of

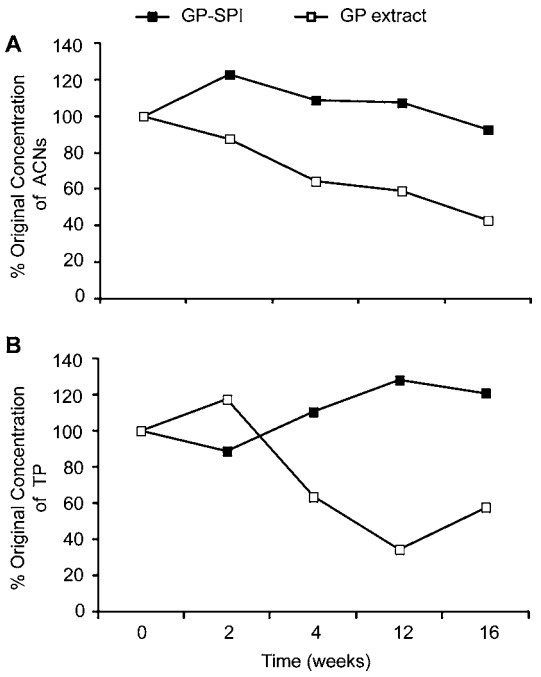


**Figure 2.** Stability of Concord grape polyphenols bound and unbound to SPI. Concord grape (A) total polyphenols and (B) anthocyanins are expressed as a percentage of the original concentration measured on day 0 (before incubation at 37 °C) and after 2, 4, 12, and 16 weeks at 37 °C.

anthocyanins in the grape pomace extract declined by more than 50% (Figure 2A). Anthocyanins are known to be highly unstable at elevated temperature;[23,24] therefore, the data suggest that complexation with SPI protects them from degradation at 37 °C. Similarly after incubation at 37 °C the levels of total polyphenols in GP−SPI were stable compared to levels in the grape pomace extract (Figure 2B), suggesting that complexation with SPI has a stabilizing effect on all polyphenols. The stabilizing effect of SPI on grape polyphenols requires further evaluation at higher cooking temperatures and various processing methods, as well as in finished food products that incorporate the GP−SPI ingredient. Preliminary taste assessments indicate that the complexation of grape pomace polyphenols with SPI masks the astringent taste of polyphenols, as the SPI prevents the interaction of tannins with salivary proteins; however, this remains to be formally evaluated in a sensory panel.

**Concord Grape Pomace Polyphenols Complexed to Soy Protein Isolate Have Hypoglycemic Activity in Mice.** We have previously demonstrated that polyphenols from Concord grape juice can be sorbed to defatted soybean flour (DSF) to produce a grape polyphenol−enriched DSF ingredient that has acute hypoglycemic activity in obese hyperglycemic mice.[9] We therefore tested the hypoglycemic effects of GP−SPI in the same model to determine if polyphenols extracted from pomace show similar biological activity. SPI or GP−SPI containing 5% total polyphenols from grape pomace was formulated in 75% Labrasol as vehicle (Veh) and administered to mice at the indicated doses. An aqueous solution of metformin (300 mg/kg) was administered as positive control (Figure 3). Before treatment mice in each

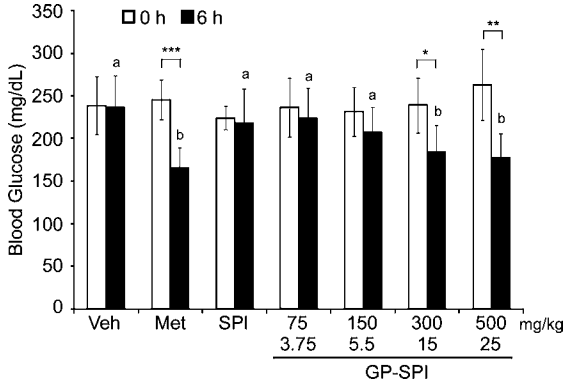


**Figure 3.** Hypoglycemic activity of GP−SPI. Blood glucose levels of C57BL/6 mice before (0 h) and 6 h after treatment with 75% Labrasol (Veh), metformin (Met, 300 mg/kg), SPI (500 mg/kg), or GP−SPI (75, 150, 300, or 500 mg/kg). The second row of numbers represents the amount of total polyphenols (TP, mg/kg) delivered with each dose of GP−SPI. Each bar represents the mean ± SD ($n$ = 6−15) of data combined from three independent experiments. Significance between groups before or after treatment was determined by ANOVA followed by Tukey unequal $n$ test. No difference was detected between groups at 0 h ($p$ = 0.32). At 6 h, different letters above bars indicate significant difference between groups ($p$ < 0.05). Significance was confirmed within groups (before and after treatment) by two-tailed, paired $t$ test: (∗) $p$ < 0.05; (∗∗) $p$ < 0.01; (∗∗∗) $p$ < 0.001.

group had similar levels of blood glucose (ANOVA, $p$ = 0.32); however, there were significant differences in blood glucose between the groups 6 h after treatment (ANOVA, $p = 4.2 \times 10^{-5}$). Compared to vehicle or SPI controls, mice administered 300 and 500 mg/kg doses of GP−SPI containing 15 or 25 mg/kg doses of grape polyphenols, respectively, had significantly lower blood glucose levels similar to the metformin treated group (Tukey unequal $n$ test). Within-group analysis (before vs after treatment) confirmed that the 300 and 500 mg/kg doses of GP−SPI significantly lowered blood glucose after 6 h (two-tailed $t$ test). The 75 and 150 mg/kg doses of GP−SPI did not show hypoglycemic activity (Figure 3). These data indicate that the grape pomace polyphenols in the GP−SPI matrix may be useful for blood glucose management and warrant further investigation of other parameters related to metabolic syndrome in longer term feeding studies.

The data indicate that GP−SPI retains, and possibly amplifies, the health benefits of grape polyphenols while excluding the glycemic sugars present in fresh and processed grapes. Normally, plant foods, such as berries, are rich in polyphenols and sugars but low in proteins, while plant foods rich in proteins, such as legume products, are low in polyphenols and sugars. GP−SPI stably combines and co-delivers proteins and polyphenols without sugars. In our informal taste assessments we have found that complexing grape pomace polyphenols to protein reduces the astringent

flavor of the extract; however, taste assessments would be most meaningful after incorporation into food products. GP−SPI may be formulated into foods or dietary supplements, such as bars, nonclear beverages, or baked goods. Such dietary intervention offers a practical approach to address the growing global incidence of metabolic disorders such as metabolic syndrome and type II diabetes;[25] however, properly controlled and executed clinical trials are needed to demonstrate the value of GP−SPI as a therapeutically useful functional food ingredient.

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### Funding

This work was supported in part by SBIR Phase I Grant 2011-33610-30489 and SBIR Phase II Grant 2012-33610-20106 from the U.S. Department of Agriculture.

### Notes

The authors declare the following competing financial interest(s): D.E.R., M.A.L., and I.R. have equity in Nutrasorb LLC, which has interest in developing polyphenol sorption technology.

## ACKNOWLEDGMENTS

We thank Brandon Mirda for technical assistance. D.E.R. designed the experiments, performed statistical analysis, and wrote the manuscript. P.K. did animal experiments. C.G.K. did MALDI-TOF and LC−MS analysis. K.M. performed optimization and stability studies. All authors read and approved the final manuscript.

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