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Ozone Elicited Phenolic Bioactives in Grapes and Health Relevant Screening Targeted for Type 2 Diabetes using In Vitro Assay Models

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Abstract

Grapes and grape derived products such as beverages are excellent sources of phenolic antioxidants with human health relevant medicinal properties. These phenolic bioactives with antioxidant function can be screened and targeted for the dietary management of oxidative stress-linked chronic diseases, such as early stages of type 2 diabetes. The aim of this study was to enhance phenolic antioxidant-linked medicinal properties in grapes (*Vitis vinifera* L.) through pre-harvest ozone treatment. Further the goal was to improve its bioactive functionality that can be potentially targeted for post-prandial glucose management through dietary intake. In this study, eight grape cultivars were treated with ozone at pre-harvest stage. Following harvest, cold water extracts of grapes were evaluated for total soluble phenol content, phenolic profile, antioxidant capacity, and α-amylase, and α-glucosidase enzyme inhibitory activities using *in vitro* assay models. Overall, all red grape cultivars and one white grape cultivar (Vignoles) showed high total soluble phenolic content and antioxidant activity when compared with other white grape cultivars. Ozone treatment significantly enhanced total soluble phenolic content in Vignoles and Frontenac grape cultivars. High α-amylase and high α-glucosidase enzyme inhibitory activities were also found in all grapes with Vignoles having the highest inhibitory activity even at one-fifth dilution. In this study, Vignoles, St Croix, Frontenac, De Chaunac, and Marechal Foch grape cultivars showed high phenolic bioactive-linked functionalities in *in vitro* assay models and have the potential to be targeted as medicinally active foods and ingredients for management of early stages type 2 diabetes. Further, these results suggest that stress induction with ozone at pre-harvest stage has potential to improve such phenolic bioactive-linked medicinal properties in specific grape cultivars like Vignoles and Frontenac.

INTRODUCTION

Grapes are grown worldwide and are consumed either as a table fruit or as a processed non-alcoholic or alcoholic beverages (wine). The global production of table grapes is expected to be around 21.0 million metric tons with the production at 984,000 tons in the United States (USDA, 2016). Among the 59 cultivars of commercial grapes that

are grown and consumed, some of the popular cultivars in the United States are St Croix, Edelweiss, Frontenac, La Crescent, Marquette, Marechal Foch, St Pepin and Concord (USDA, 2011). The popularity of grapes among consumers is growing rapidly due to its flavor, taste, and diverse human health relevant medicinal properties. Grapes are a rich source of human health relevant phenolic compounds which have diverse medicinal values and varies widely with type of grape (white and red), genotype, geographical origin, oenological practices, and exposure to biotic or abiotic stresses (Frémont, 2000). The main difference between red and white grape varieties is that red grapes have higher anthocyanin content than white grapes. However, in both cases, grape skins are a rich source of anthocyanins, hydroxycinnamic acids, flavanols and flavonol glycosides (Kammerer et al., 2004, Ramchandani et al., 2010).

The human health relevant phenolic compounds of grapes are secondary metabolites that have diverse roles in plant growth, lignification, pigmentation, pollination and biotic and abiotic stress tolerance (Fraga et al., 2010, Fremont, 2000). Further, these same phenolic compounds are effective antioxidants and can be utilized in dietary strategies to counter oxidative-stress linked diseases such as early stages of type 2 diabetes and cardiovascular diseases (Leonard et al., 2003). Phenolic metabolites of grapes can quench super oxide radical O_2^- and hydrogen peroxide H_2O_2 and can also inhibit prostaglandin production which in turn reduces inflammation (Martinez and Moreno, 2000). Phenolics also have a dual role in improving mitochondrial function and providing protection against diet induced obesity and insulin resistance (Lagouge et al., 2006). The major mechanisms by which phenolics can quench free radicals include non-specific (electron transfer) and specific interactions based on structural conformation of phenolic acids which allow these compounds to interact with target proteins, including enzymes, membrane proteins, transcription factors and membrane receptors that are involved in countering chronic oxidative stress (Fraga et al., 2010). Grapes also have a low glycemic index and when consumed have shown to reduce hyperglycemia, and improve pancreatic β cell function (Zunino, 2009). Further, previous research has found that grapes and beverages derived from grapes have inhibitory activity against enzymes like α-amylase and α-glucosidase and thus has the potential to reduce postprandial hyperglycemia (Hogan et al., 2010, Kwon et al., 2008). Therefore, grapes and grape derived non-alcoholic beverages can be screened and targeted as medicinally active plant and food source for the dietary management of early stages of type 2 diabetes and associated complications.

Several strategies involving plants endogenous defense responses against biotic and abiotic stresses (UV-B, Ozone, and chemical) has been identified and utilized to improve phenolic biosynthesis and antioxidant activity in grapes during pre- and post-harvest stages (Gonzalez-Barrio et al. 2006, Portu et al. 2015, Ruiz-Garcia et al. 2012, Wang et al. 2013). The major goals of these previous studies were to enhance phenolic biosynthesis to improve biotic and abiotic stress tolerance and fruit quality of grapes. But same strategy involving stress-induction can be used to improve human health relevant phenolic antioxidant profile and subsequent medicinal properties in grapes and in foods derived from grapes. Therefore, the major aim of this study was to improve health relevant phenolic bioactives and antioxidant activity in eight grape cultivars (both red and white) through ozone treatment and to compare it with untreated grapes. Further, the specific goal was to evaluate the potential impact on phenolic bioactive enrichment in grapes by screening for human health benefits using *in vitro* assay models for targeting dietary management of early stages of type 2 diabetes.

MATERIALS AND METHODS

Materials. Eight grape cultivars were used in this study that included both red and white grapes. The red grape cultivars included Marechal Foch, Frontenac, De Chaunac and St Croix while the white grape varieties were La Crescent, Edelweiss, Brianna and Vignoles. Porcine pancreatic alpha–amylase (EC 3.2.1.1), rat intestinal alpha–glucosidase (EC

3.2.1.20), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and phenolic acid standards were purchased from Sigma Chemical Co (St. Louis, MO).

Treatments. The field experiments with red and white grape cultivars were carried out at Mac's Creek Winery & Vineyards, Lexington, Nebraska in 2014. Each of the grape cultivars were arranged into ozone and untreated (control) groups. The sample groups were selected by row blocks which consisted of three rows for each group having the control group in between the ozone group in order to minimize drift or overlap between the groups. For the ozone treatment, ozone was sprayed onto the grapes at the rate of 25 gallons per acre, once every 10 days. A total of six rounds of ozone spraying were done with the help of an air blast mist sprayer. The treated (ozone) and untreated grapes were stored and transported to North Dakota State University at -20°C prior to phenolic bioactive and functionality analysis.

Sample Extraction. For the cold-water extraction procedure, 40 g of each of the grape cultivars were weighed and each sample was then homogenized with 100 mL of distilled water using a Waring blender for 5 minutes at low speed. The homogenized samples were then centrifuged at 8,500 rpm for 20 minutes after which the supernatant was collected and then re-centrifuged at the same rpm for 15 minutes. The cold-water extracts were stored at 4°C during the period of the biochemical analysis.

Total Soluble Phenolics Assay. A modified protocol was used to measure the total soluble phenolics based on Shetty et al. 1995. In this protocol, 0.5 mL of sample extracts were added to their respective test tubes. For the control 0.5 mL of distilled water was added instead of sample. This was followed by the addition of 0.5 mL of distilled water, 1 mL of 95% ethanol to all the tubes. Then 0.5 mL of 50% (v/v) Folin- Ciocalteu reagent was added to each tube. This was followed by the addition of 1 mL 5% sodium carbonate. The contents in each tube were mixed using a vortex and were incubated under dark conditions for 60 minutes at room temperature. After incubation the absorbance was checked using a spectrophotometer (Genesys, Thermo Fisher Inc.) set at 725nm. The absorbance values were converted to total soluble phenolic content and expressed in milligram per gram of fresh weight with the help of standard curve that was established using different concentrations of gallic acid in 95% ethanol.

Antioxidant Capacity through DPPH (2, 2, diphenyl-1-picrylhydrazyl) Radical Scavenging Assay. For this assay as described by Kwon et al. (2006), 1.25 mL of 60 mM DPPH (in 95% ethanol) was added to 0.25 mL of sample. The tubes were gently tapped to ensure mixing and were incubated for 5 minutes, after which they were centrifuged for 1 minute at 13,000 rpm and the absorbance of the supernatant was measured at 517nm using a UV spectrophotometer (Genesys). Each sample had a corresponding control which contained 0.25 mL of 95% ethanol. Based on the absorbance readings, the percentage of inhibition was calculated using the formula:

$$\% \text{ inhibition} = (\text{Absorbance}^{\text{control}} - \text{Absorbance}^{\text{extract}}) / \text{Absorbance}^{\text{control}} \times 100$$

Alpha-Glucosidase Inhibitory Activity. The assay followed here was based on the protocol by Worthington Enzyme Manual (1993) with some modifications taken from McCue et al. (2005*)*. A total of 10 µL, 25 µL and 50 µL of each sample extract was pipetted into 96 well micro titer plates and used as undiluted, half diluted and 1/5 diluted extracts respectively. The half dilution and 1/5 dilutions were made up to a total of 50 µL in volume by adding 25 µL and 40 µL of 0.1M potassium phosphate buffer (pH 6.9), respectively. Each sample extract had a corresponding control of 50 µL of phosphate buffer. Finally, the volume in all the wells was made up to 100 µL by the addition of 50 µL of phosphate buffer in each well including the controls. Then 100 µL of 0.1M potassium phosphate buffer (pH 6.9) containing α glucosidase enzyme (1 U/mL) was added to each well and incubated at 25°C for 10 minutes. After this 50 µL of 5 mM p-nitrophenyl-α-D- glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were then incubated at 25°C for 5 min. Absorbance readings were taken before (0 minute) and after (5 minute) incubation

period using a micro plate reader (Thermomax, Molecular device Co., Virginia, USA) set at 405nm. The percentage of α glucosidase inhibitory activity was calculated based on the absorbance readings of the sample extract and their respective controls using the formula:

$$\% \text{ inhibition} = (Abs^{control}\ 5min - Abs^{control}\ 0\ min) - (Abs^{extract} 5min - Abs^{extract} 0\ min) / Abs^{control}\ 5min - Abs^{control}\ 0\ min) \times 100$$

Alpha-Amylase Inhibitory Activity. The protocol followed here was taken from the Worthington Enzyme Manual (1993). Similar to α-glucosidase, undiluted, half diluted and 1/5th diluted extract samples were used for this assay. The dilutions were done using distilled water. The buffer used was 0.1M sodium phosphate (pH 6.9) with 0.006M sodium chloride added to it. Around 500 µL of each sample extract was added to test tubes while the control tubes had 500 µL of buffer only. Additionally, each sample extract had a corresponding sample blank tube which contained 500 µL of the sample extract. Then of 500 µL of porcine pancreatic amylase (0.5 mg/ mL buffer) was added to all the tubes with the exception of the sample blank tubes, and incubated at 25°C for 10 minutes. After incubation, 500 µL of 1% starch (1 g/100 mL buffer) was added to all the tubes and incubated for 10 minutes. The reaction was then stopped by the addition of 1 mL of 3, 5 dinitro salicylic acid and the tubes were placed in a boiling water bath for 10 minutes after which the tubes were taken out and cooled to room temperature. The reaction mixture in the tubes was then diluted by adding 10 mL of distilled water and the absorbance was measured at 540 nm. The percentage of inhibition of α amylase enzyme was calculated using the formula:

$$\% \text{ inhibition} = Abs^{control} - (Abs^{extract} - Abs^{sample\ blank})/ Abs^{control} \times 100$$

HPLC Analysis of Phenolic Profiles. Two milliliter of fruit extracts were filtered through a 0.2 µm filter and then centrifuged for 5 min. A 5 µL volume of sample was injected using Agilent ALS 1200 auto sampler into Agilent 1260 series HPLC (Agilent Technologies, Palo Alto, CA equipped with DAD 1100 diode array detector). The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% over the next seven minutes, then decreased to 0% for the next 3 min and was maintained for the next 7 min (total run time, 25 min). The analytical column was used was Agilent Suppelco SB-C18 250 x 4.6 mm i.d., with packing material of 5 µm particle size at a flow rate of 0.700 mL/min at ambient temperature. During each run the chromatogram was recorded at 254 nm and 306 nm and integrated using Agilent Chemstation enhanced integrator. Pure standards of gallic acid, protocatechuic acid, catechin, chlorogenic acid, caffeic acid, quercetin derivatives, resveratrol and p-coumaric acid (purchased from Sigma Chemical Co., St. Louis, MO) in 100% methanol were used to calibrate the standard curve and retention times.

Statistical Analysis. The complete biochemical analysis was repeated four times. Analysis at every time point from each experiment was carried out in triplicates. Means, standard errors, and standard deviations were calculated from replicates within the experiments and analyses were done using Microsoft Excel XP. The data were analyzed with analysis of variance (ANOVA) of Statistical Analytical Software (SAS version 9.4; SAS Institute, Cary, NC). Differences among cultivars and treatments and cultivar × treatments interactions were determined by the Tukey's least mean square test at the 0.001 probability level.

RESULTS

Total Phenolic Content. The total soluble phenolic content of grape extracts was determined using Folin-Ciocalteau method. Overall, significantly higher ($p<0.001$) total soluble phenolic content was observed in red grapes when compared to white grape cultivars, with exception in Vignoles (Fig 1). Significant differences ($p<0.001$) in total soluble phenolic content between cultivars, treatments, and cultivar × treatment interactions were found in this study (Table 1). Ozone treatment was most effective for the red grape cultivar Frontenac, which had

significantly higher total soluble phenolic content of 1.37 mg GAE/ g F.W. ($p<0.001$) when compared to the untreated grapes.

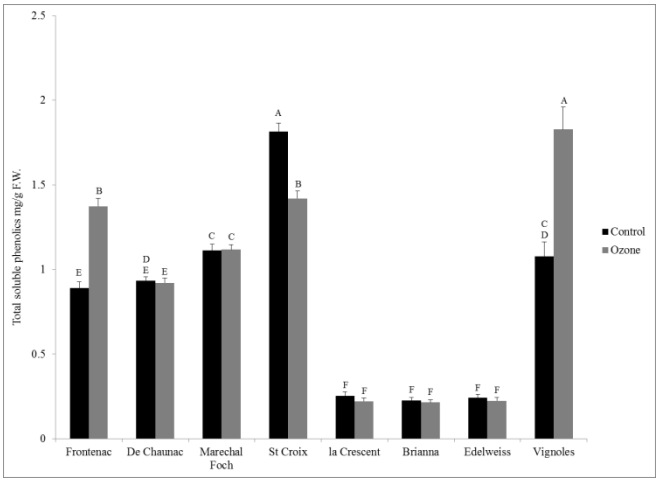

Figure 1. Total soluble phenolic content (mg GAE/ g F.W.) of eight red and white grape cultivars with two different pre-harvest treatments (control and ozone). Different capital letters represent significant differences between cultivar × treatment interactions at $p<0.001$ probability levels.

Similarly, among the white grape cultivars, ozone treatment enhanced the total soluble content of the Vignoles cultivar nearly two-fold (1.83 mg GAE/g F.W) which was significant when compared to untreated grapes ($p<0.001$).

Table 1. Analysis of variance (ANOVA) for total soluble phenolic content (TSP), antioxidant activity (AA), α-amylase, and α-glucosidase inhibitory activity between cultivars (eight), treatments (two), and cultivar × treatment interactions.

		TSP	AA	α-amylase Inhibitory Activity			α-glucosidase Inhibitory Activity		
	df			Undiluted	1:2 dilution	1:5 dilution	Undiluted	1:2 dilution	1:5 dilution
Cultivar	7	***	***	***	***	***	***	***	***
Treatment	1	***	***	*	***	*	**	***	***
Cultivar x treatment	7	***	***	NS[a]	*	**	***	***	***

*P<0.05; ** p<0.01; *** p<0.001; a Not Significant

Among the red grape cultivars, untreated St Croix had the highest total soluble phenolic content of 1.81 mg GAE/ g F.W., which was significantly higher ($p<0.05$) than the ozone treated grape. White grape cultivars such as La Crescent, Brianna, and Edelweiss had lower baseline total soluble phenolic content ranging from 0.19 to 0.27 mg GAE/g F.W. Ozone treatment also did not result in any improvement in total soluble phenolic content in these white grape cultivars.

*Total Antioxidant Activity***.** The total antioxidant activity of the grape cultivars was measured by their ability to quench the DPPH radical. Similar to the total soluble phenolic content, significant differences ($P<0.001$) in total antioxidant activity between cultivars, treatments, and cultivar × treatment interactions were also observed (Table 1). In general, the antioxidant activity was significantly higher in the red grape cultivars (80-90% DPPH inhibition) when compared to the white grape cultivars (12-25% DPPH inhibition) (Fig 2). However, among the white grape cultivars, Vignoles had very high antioxidant activity (91% DPPH inhibition) which is in the same range as red grape cultivars. Among red grape cultivars, De Chaunac with ozone treatment had significantly ($p<0.001$) higher antioxidant activity when compared to the untreated grapes. On the contrary, the untreated Marechal Foch and Frontenac cultivar had significantly higher antioxidant activity (88%) when compared to the ozone treated grapes ($p<0.001$).

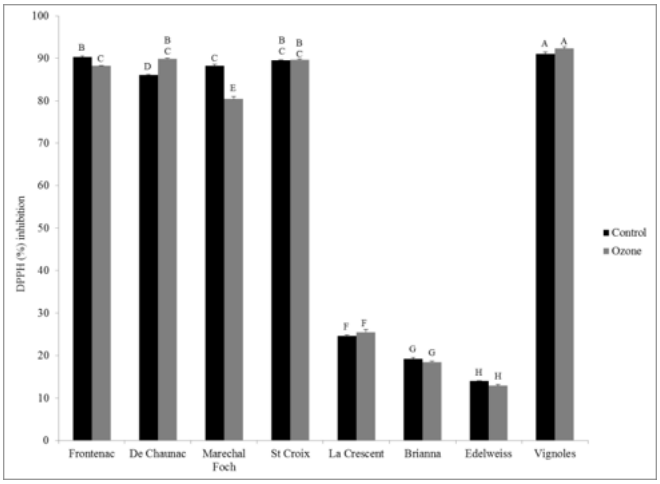

Figure 2. Total antioxidant activity (DPPH radical scavenging % inhibition) of eight red and white grape cultivars with two different pre-harvest treatments (control and ozone). Different capital letters represent significant differences between cultivar × treatment interactions at $p<0.001$ probability levels.

Alpha-Amylase Inhibitory Activity. To determine the role of grape bioactives for improving glucose metabolism, α-amylase inhibitory activity of all

grape cultivars with and without ozone treatment was determined through model *in vitro* assay. Significant differences ($p<0.001$) in α-amylase inhibitory activity between cultivars were observed in all dilutions (Table 1). In undiluted samples, no significant differences between cultivar × treatment interactions were observed, while in ½ ($p<0.05$) and 1/5th dilutions ($p<0.01$) significant differences between cultivar × treatment interactions were observed (Table 1). In this study all grape cultivars (both red and white grapes) showed very high α-amylase inhibitory activity (70-100% inhibition) with significant dose responses (Fig 3 A, B, C). Among all cultivars, Vignoles exhibited very high α-amylase inhibitory activity even at the 1/5th of dilution (88%), which was significantly higher when compared with all other red and white grape cultivars ($p<0.001$). Ozone treated Vignoles grape showed higher trend of α-amylase inhibitory activity in undiluted and ½ diluted sample but was not statistically significant when compared with untreated grapes. Ozone treated Frontenac grape showed significantly higher α-amylase inhibitory activity when compared with untreated grapes ($p<0.001$) at ½ dilution. At 1/5th dilution untreated Edelweiss had significantly higher α-amylase inhibitory activity when compared to ozone treated grapes ($p<0.001$).

Alpha-Glucosidase Inhibitory Activity. The inhibitory activity of α-glucosidase which is a key enzyme involves in human glucose metabolism was also determined using *in vitro* assay model. Significant differences in α-glucosidase inhibitory activity between cultivar × treatment interactions ($p<0.001$) were observed in all dilutions (Table 1). Similar to α-amylase inhibitory activity, all red and white grape cultivars had very high α-glucosidase inhibitory activity in this study (Fig 4 A, B, C). Like soluble phenolic content, antioxidant activity, and α-amylase inhibitory activity, white grape cultivar Vignoles had very high α-glucosidase inhibitory activity even at 1/5th dilution (99%), which was significantly higher than other red and white grape cultivars ($p<0.001$). Among red grape cultivars, higher α-glucosidase inhibitory was found in Marechal Foch, and St. Croix ($p<0.001$).

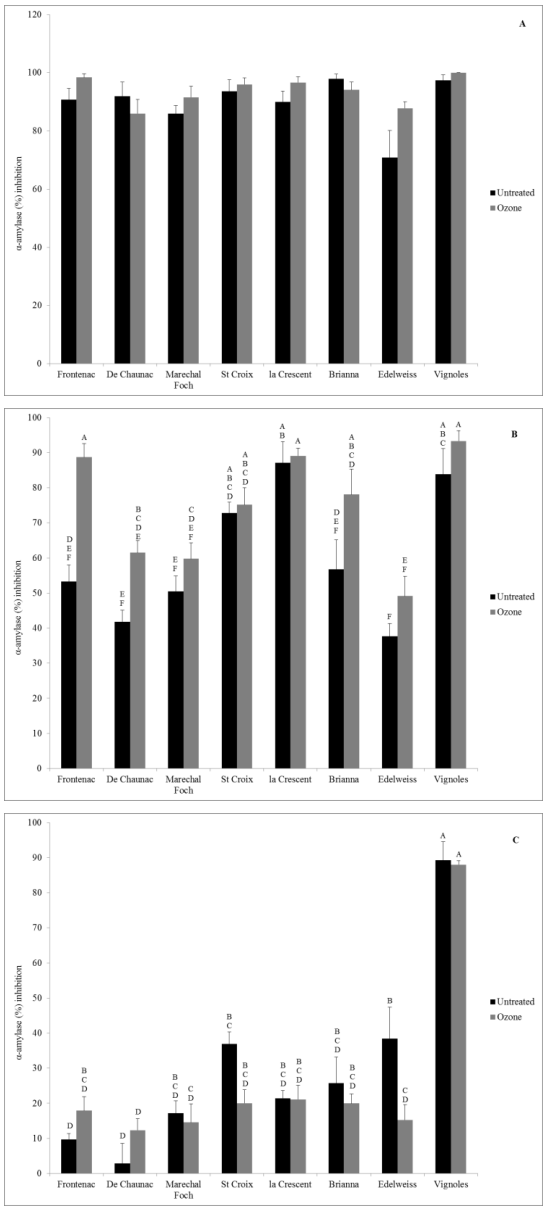

Figure 3. Alpha-amylase enzyme inhibitory activity (% inhibition) with three different doses (undiluted A, ½ dilution B, and 1/5th dilution C) of eight red and white grape cultivars with two treatments (control and ozone). Different capital letters represent significant differences between cultivar × treatment interactions at $p<0.05$ and $p<0.01$ probability levels. No significant differences in α-amylase inhibitory activity between cultivar × treatment interactions were observed in undiluted samples.

Positive correlation between total soluble phenolic content and α-glucosidase inhibitory activity was observed in all grape cultivars. Vignoles, St Croix, Frontenac, De Chaunac, and Marechal Foch showed high α-glucosidase inhibitory activity, while La

Crescent, Brianna, and Edelweiss had moderate α-glucosidase inhibitory activity at 1/5th dilution. Untreated Edelweiss grape resulted in significantly higher α-glucosidase inhibitory activity when compared to ozone treated Edelweiss grape at undiluted and at 1/5th dilution ($p<0.001$).

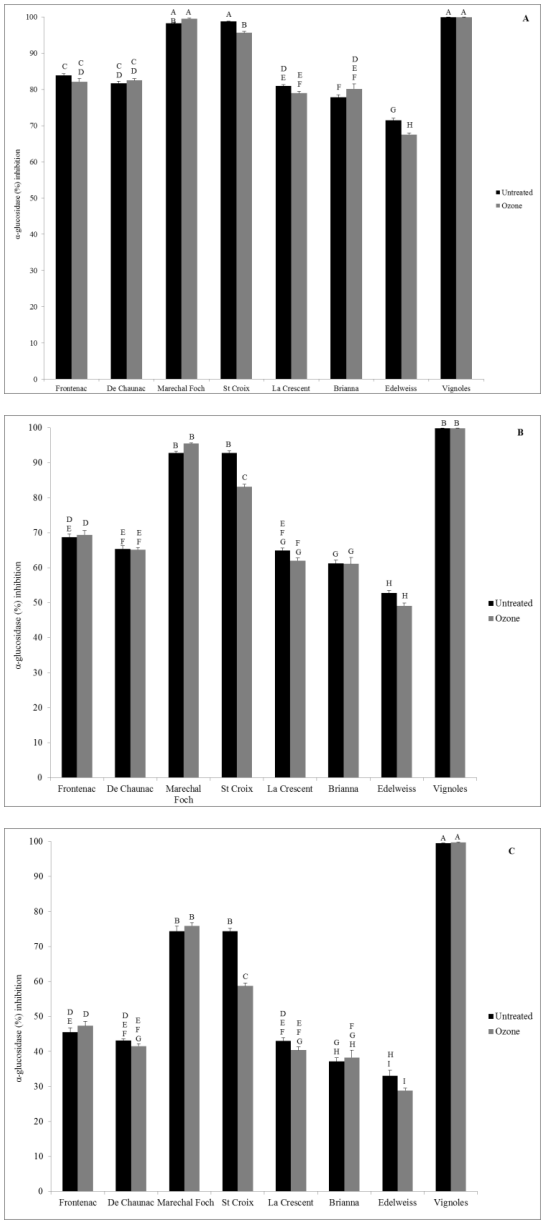

Figure 4. Alpha-glucosidase enzyme inhibitory activity (% inhibition) with three different doses (undiluted A, ½ dilution B, and 1/5th dilution C) of eight red and white grape cultivars with two different pre-harvest treatments (control and ozone). Different capital letters represent significant differences between cultivar × treatment interactions at $p<0.001$ probability levels.

Phenolic Profile through HPLC. High performance liquid chromatography (HPLC) analysis was carried out to determine phenolic acid content in red and white grape cultivars. In this study, catechin and gallic acid were the two major phenolic acids found in red and white grape cultivars except for Brianna and Edelweiss, whereas only catechin was detected (Table 2). In all red and white grape cultivars concentrations of catechin were 2-3 folds higher than the gallic acid concentrations. Similar to total soluble phenolic content the catechin concentrations in white grape cultivars such as La Crescent, Brianna, and Edelweiss were significantly lower compared to all red grape cultivars and Vignole white grapes. No significant differences in catechin and galllic acid concentrations were found with ozone treatment. A higher concentration of catechin was found in ozone treated La Crescent grape when compared with untreated grape.

Table 2: Concentrations (µg/mL) of two major phenolic compounds (catechin and gallic acids) determined using high performance liquid chromatography (HPLC) method of eight grape cultivars with two treatments (control and ozone).

Cultivar	Treatment	Catechin (µG/mL)	Gallic Acid (µG/mL)
Frontenac	Control	13.11	4.99
	Ozone	12.02	5.02
De Chaunac	Control	11.63	4.88
	Ozone	12.92	5.52
Marechal Foch	Control	14.07	4.83
	Ozone	13.07	5.32
St Croix	Control	12.77	6.19
	Ozone	11.85	5.75
La Crescent	Control	5.97	0.55
	Ozone	8.42	0.55
Brianna	Control	6.67	ND[a]
	Ozone	6.21	ND
Edelweiss	Control	5.14	ND
	Ozone	5.05	ND
Vignoles	Control	16.60	6.92
	Ozone	16.69	6.86

[a] Not Detected

DISCUSSION

Rich and diverse phenolic bioactive profiles in grape offer significant health benefits and can be targeted as medicinally active plant food to manage oxidative

stress-induced diseases such as non-communicable chronic diseases, especially for type 2 diabetes and associated macro-vascular complications such as cardiovascular disease (Leonard et al. 2003, Yang and Xiao 2013). The medicinal properties of grape and in grape derived beverages are mostly due to the phenolic bioactive profile and high antioxidant activity (Georgiev et al. 2014). But the phenolic profile as well as antioxidant activity varies widely among different red and white grape cultivars and results in varying quality of fruit as well as alcoholic and non-alcoholic beverages (Fremont 2000). Further, the growing conditions, horticultural practices, environment, and post-harvest storage also dictate the health relevant bioactive profile of the grape fruits and can lead to heterogeneous fruit and wine quality. In this study, significant differences in phenolic antioxidant-linked functionalities for potential dietary management of early stages of type 2 diabetes between red and white grape cultivars were observed. But among all grape cultivars, Vignoles (white grape cultivar) showed very high phenolic bioactive-linked functionalities for potentially improving glucose metabolism based on indications from *in vitro* assays when compared to all other white and red grape cultivars. Positive correlation between total soluble phenolic content, antioxidant activity, α-glucosidase, and α-amylase enzyme inhibitory activity was also observed in Vignoles.

Overall, higher total soluble phenolic content and higher berry antioxidant capacity was observed in red grape cultivars when compared with white grape cultivars. Lower concentration of anthocyanin and other phenolic acids were observed in white grapes and in white wines in previous studies (Kammerer et al. 2004, Kwon et al. 2008, Vinson and Hontz 1995). But white grape cultivar, Vignoles showed similar level of total soluble phenolic content and higher antioxidant activity like other red grape cultivars evaluated in this study. The most interesting finding of this study was the significant increase in total soluble phenolic content in ozone treated Vignoles grape. Increased concentrations of several anthocyanins and flavonols were observed in grapes with foliar application of phenylalanine and urea in grape vines (Portu et al. 2015). Similarly, higher accumulation of viniferins was found in white grape 'Superior' with gas ozone treatments (Gonzalez-Barrio et al. 2006). Although ozone treatment did not result in increasing soluble phenolic content in all grape cultivars, this strategy might be effective for selected cultivars such as white grape Vignoles and red grape Frontenac. Therefore, from the findings of this study we can hypothesize that the response to stress-induction through treatments such as ozone for improving phenolic antioxidant is very much dependent on grape genotypes along with other phenotypic factors. Overall, all red grape cultivars and Vignoles white grape had very high antioxidant (DPPH radical scavenging activity) activity and thus showed potential to be utilized as dietary antioxidants to counter oxidative stress-linked chronic diseases such as type 2 diabetes. Higher antioxidant activity (DPPH) of crude polyphenolic extracts of seedless and seeded Indian grapes was also found in a previous study (Ramchandani et al. 2010).

Similar to total soluble phenolic content and total antioxidant activity, higher inhibitory activity of enzymes such as α-glucosidase, and α-amylase was also observed in all red and white grape cultivars (without dilution). Although Vignoles white grape had highest α-glucosidase, and α-amylase enzyme inhibitory activities, these did not improve with ozone treatment. One possible reason for not finding significant differences with stress induction is due to very high baseline value of both α-glucosidase, and α-amylase enzyme inhibitory activities in this white grape cultivar. Even after one-fifth dilution Vignoles had high α-glucosidase, and α-amylase inhibitory activities. Similarly, other red grape cultivars such as St. Croix, Marechal Foch and Frontenac showed high α-glucosidase inhibitory activity. Improvement of α-amylase inhibitory activity was found in ozone treated Frontenac grape and it positively correlated with total soluble phenolic content. Therefore, the high α-amylase enzyme inhibitory activity of Frontenac and Vignoles might be due to their high soluble phenolic content. Therefore, Vignoles white grape cultivar along with other red grape cultivars such as St. Croix, Marechal Foch and Frontenac can

be potentially targeted for dietary management of early stages of type 2 diabetes as reflected from these *in vitro* assay models. Higher α-glucosidase inhibitory activity was observed previously in red wines (Kwon et al. 2008). Similarly, antidiabetic properties in polyphenolic extracts of red wine was also observed in rat model (Al-Awwadi et al. 2004). High phenolic antioxidant content and high enzyme inhibitory activity in grapes relevant for improving glucose metabolism would allow this fruit to be targeted as a medicinally active plant and as a dietary choice to improve post-prandial glucose metabolism. Not only is this relevant for maintaining glucose homeostasis but due to high and rich antioxidant profiles, grape and grape derived food products can be targeted to counter chronic oxidative stress and inflammation associated with type 2 diabetes.

In this study, we only found catechin and gallic acid in all red and white grape cultivars. Previous research reported catechin, gallic acid, quercetin, coumaric acid, caffeic acids and resveratrol in different grape cultivars (Kennedy 2008, Pastrana-Bonilla et al. 2003). Use of different organic solvents, extraction methods, concentrations, and HPLC protocols might have resulted in the detection of other phenolic acids in these previous studies. Catechin and gallic acid were two major phenolic acids observed in this study and are known to have significant health benefits when consumed as part of the diet (Frankel et al, 1995). White grape cultivars such as La Crescent, Brianna, and Edelweiss had lower concentrations of catechin and this observation positively correlated with phenolic antioxidant linked functionalities in these cultivars. Therefore, the bioactive functionalities relevant for dietary management of type 2 diabetes might be due to the concentration and compositions of phenolic acids in red and white grape cultivars. Overall, this study showed that red grape cultivars and white grape cultivar such as Vignoles with higher phenolic antioxidant-linked functionalities are an ideal choice to be targeted as medicinally active food source for dietary management of early stages type 2 diabetes and to potentially mitigate associated oxidative stress-linked complications. The impact of stress induction to improve phenolic antioxidant-linked functionalities in grape cultivars depends on their genetic profile and variability in the growing conditions.

CONCLUSION

Grapes are one of the most popular fruits in the world and are rich in human health relevant medicinal properties. This rich composition of phenolic-linked medicinal properties in grape potentially offers significant health benefits and can be targeted to manage oxidative stress-linked chronic diseases such as type 2 diabetes. Different strategies involving endogenous defense response pathways of plants have been developed to improve phenolic antioxidant profile in fruits and other food crops. Ozone treatments provide significant protection against biotic and abiotic stresses in plants including grapes. Same strategy can be used to improve medicinal properties in fruits during pre- and post-harvest stages. In this study we observed very high phenolic antioxidant-liked functionalities relevant for dietary management of type 2 diabetes in red and white grape cultivars using *in vitro* assay models. Improvement of these phenolic antioxidant-linked functionalities with ozone treatment was also observed in selected red and white grape cultivars such as Frontenac and Vignoles. Among all red and white grape cultivars, Vignoles had very high phenolic antioxidant-linked functionalities and could be used for future animal, clinical and epidemiological studies targeting to improve human glucose metabolism. This present study provides initial insights and strong foundation to utilize stress-induced strategy such as ozone treatment to improve human health relevant medicinal properties in selected red and white grape cultivars. Future studies with different cultivars, other stress treatments, and under different locations are needed to validate the findings of this study and to advance this innovative concept. This innovative strategy will help grape growers and wineries to improve medicinal properties and shelf-life of grape fruits and foods and beverages derived from grapes.

REFERENCES

Al-Awwadi, N., J. Azay, P. Poucheret, G. Cassanas, M. Krosniak, C. Auger, F. Gasc, J-M. Rouanet,

G. Cros, and P-L. Teissedre. 2004. Antidiabetic activity of red wine polyphenolic extract, ethanol, or both in streptozotocin-treated rats. J Agric. Food Chem. 52:1008–1016.

Fraga, C.G., M. Galleano, S.V. Verstraeten, and P.I. Oteiza. 2010. Basic biochemical mechanisms behind the health benefits of polyphenols. Mol. Aspects Med. 31:435–445.

Frankel, E. N., A.L. Waterhouse, and P.L. Teissedre. 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. J Agric. Food Chem. 43: 890-894.

Frémont, L. 2000. Biological effects of resveratrol. Antioxid. Redox Signal 66:663-673.

Georgiev, V., A. Ananga, and V. Tsolova. 2014. Recent advances and uses of grape flavonoids as nutraceuticals. Nutrients 6:391–415.

Gonzalez-Barrio, R., D. Beltran, E. Cantos, M.I. Gil, J.C. Espin, and F.A.Tomas-Barberan. 2006. Comparison of ozone and UV-C treatments on the postharvest stilbenoid monomer, dimer, and trimer induction in var. 'Superior' white table grapes. J Agric. Food Chem. 54:4222–4228.

Hogan, S., L. Zhang, J. Li, S. Sun, C. Canning, and K. Zhou. 2010. Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase. Nutr. Metabolism 7:71.

Kammerer, D., A. Claus, R. Carle, and A. Schieber. 2004. Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. J Agric. Food Chem. 52:4360–4367.

Kennedy, J.A. 2008. Grape and wine phenolics: Observations and recent findings. Ciencia e investigación agraria 35:107-120.

Kwon, Y., E. Apostolidis, and K. Shetty. 2008. Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. J. Food Biochem. 32:15–31.

Lagouge, M., C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver, and J. Auwerx. 2006. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell 127:1109–1122.

Leonard, S.S., C. Xia, B. Jiang, B. Stinefelt, H. Klandorf, G.K. Harris, and X. Shi. 2003. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochem. Biophys. Res. Commun. 309:1017–1026.

Martinez, J. and J.J. Moreno. 2000. Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. Biochem. Pharmacol. 59: 865–870.

McCue, P., Y-I. Kwon, and K. Shetty. 2005. Anti-amylase, anti-glucosidase and angiotensin I-converting enzyme potential of selected foods. J. Food Biochem. 29:278–294.5.

Pastrana-Bonilla, E., C.C. Akoh, S. Sellappan, and G. Krewer. 2003. Phenolic content and antioxidant capacity of muscadine grapes. J. Agric. Food Chem. 51:5497-5503.

Portu, J., I. Lopez-Alfaro, S. Gomez-Alonso, R. Lopez, and T. Garde-Cerdan. 2015. Changes on grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea. Food Chem. 180:171–180.

Ramchandani, A.G., R.S. Chettiyar, and S.S. Pakhale. 2010. Evaluation of antioxidant and anti-initiating activities of crude polyphenolic extracts from seedless and seeded Indian grapes. Food Chem. 119:298-305.

Ruiz-Garcia, Y., I., Romero-Cascales, R. Gil-Munoz, J.I. Fernandez-Fernandez, J. Lopez-Roca, and E. Gomez-Plaza. 2012. Improving grape phenolic content and wine chromatic characteristics through the use of two different elicitors: Methyl jasmonate versus benzothiadiazole. J. Agric. Food Chem. 60:1283-1290.

Shetty, K., O.F. Curtis, R.E. Levin, R. Witkowsky, and W. Ang. 1995. Prevention of vitrification associated with in vitro shoot culture of oregano (*Origanum vulgare*) by *Pseudomonas* spp. J. Plant Physiol. 147:447-451.

USDA 2016. Fresh Deciduous Fruit: World Markets and Trade (Apple, Grapes, & Pears) http://apps.fas.usda.gov/psdonline/circulars/fruit.pdf

USDA 2011. 2011 Grape & Wine Industry Survey https://www.nass.usda.gov/Statistics_by_State/Wisconsin/Publications/Crops/2011/WIGrapeWine.pdf

Vinson, J.A. and B.A. Hontz. 1995. Phenol antioxidant index: Comparative antioxidant effectiveness of red and white wines. J Agric. Food Chem. 43:401–403.

Wang, L., L. Ma, H. Xi, W. Duan, J. Wang, and S. Li. 2013. Individual and combined effects of $CaCl_2$ and UV-C on the biosynthesis of resveratrols in grape leaves and berry skins. J Agric. Food Chem. 61:7135–7141.

Worthington Biochemical Corp. 1993. Maltase-a-glucosidase. In: Worthington, V., (Ed.), Worthington Enzyme Manual. Freehold, pp. 261.

Yang, J. and Y.Y. Xiao. 2013. Grape phytochemicals and associated health benefits. Crit. Rev. Food. Sci. 53:1202–1225.

Zunino, S. J. 2009. Type 2 diabetes and glycemic response to grapes or grape products. J. Nutr. 139:1794S-1800S.