Journal of Medicinally Active Plants

Volume 5
Issue 2 *Vol 5 Issues 1-4*

October 2017

Instantaneous Heat Shock Treatment in Grape During Pre-Harvest Stages Enhances Phenolic-linked Medicinal Properties in Red Wine

Avani Manduri
North Dakota State University, avanimanduri@gmail.com

Dipayan Sarkar
North Dakota State University - Main Campus, Dipayan.sarkar@ndsu.edu

Marty Fischer
Agrothermal Systems, marty@agrothermalsystems.com

Chad Vargas
Adelsheim Vineyard, cvargas@adelsheim.com

Kalidas Shetty
North Dakota State University--Fargo, kalidas.shetty@ndsu.edu

Recommended Citation
Manduri, Avani; Dipayan Sarkar; Marty Fischer; Chad Vargas; and Kalidas Shetty. 2016. "Instantaneous Heat Shock Treatment in Grape During Pre-Harvest Stages Enhances Phenolic-linked Medicinal Properties in Red Wine." *Journal of Medicinally Active Plants* 5, (2):36-46.
DOI: 10.7275/R5JH3JCC
http://scholarworks.umass.edu/jmap/vol5/iss2/6

Instantaneous Heat Shock Treatment in Grape During Pre-Harvest Stages Enhances Phenolic-linked Medicinal Properties in Red Wine

Avani Manduri[1], Dipayan Sarkar[1], Marty Fischer[2], Chad Vargas[3], and Kalidas Shetty[1*]

[1]Department of Plant Sciences, North Dakota State University, ND-58103, USA,
[2]Agrothermal System, CA-94597, USA, [3]Adelsheim Vineyard, OR-97132, USA

***Corresponding author**: kalidas.shetty@ndsu.edu

Manuscript Received: March 14, 2016

Keywords: Abiotic stress, antioxidants, beverage, hyperglycemia, type 2 diabetes, medicinal properties.

ABSTRACT

Mild abiotic stress induction can potentially improve phenolic-linked antioxidant capacity and associated relevant human health medicinal properties in food crops through stimulation of endogenous defense-related metabolic responses. Grape and grape fermented products, such as red wines, are excellent sources of diverse phenolic antioxidants with medicinal value. Due to the diverse medicinal properties and the wide popularity of grapes as food and beverage, grape and grape-derived red wines are ideal candidates for the improvement of phenolic bioactive-linked functionalities through use of pre-harvest, instantaneous heat shock induction as a stress-linked stimulation.

INTRODUCTION

Among plant-based food, fruit, and fruit derived beverages (both alcoholic and nonalcoholic) are generally excellent sources of dietary phenolics and antioxidants with health benefits. Due to the rich flavor compositions and wide acceptability among consumers, fruit beverages with human health benefits are good targets for dietary management of oxidative stress-linked, non-communicable chronic diseases (NCDs). As a very popular fruit worldwide, grapes are widely consumed as table food, dried food, and as alcoholic and non-alcoholic beverages. Beyond the diverse food and nutritional values, grapes are also considered as medicinally active foods due to diverse human health benefits.

From ancient times, grapes and red wines have been a major part of traditional Mediterranean diet and have been recognized for contributing to human health, especially for improving the cardiovascular system. The largely known phenomenon "French paradox" primarily describes the incidence of lower mortality rate from cardiovascular pathologies among the French population due to higher consumption of red wine. Grapes and red wine contain large amounts of biologically active phenolic compounds, including resveratrol and other phenolic acids (Vitagilone *et al*., 2005). In the past few decades, plant phenolics have been receiving increased interest as the plant phenolics have diverse applications and relevant plant responses to biotic and abiotic stresses that improve food quality, food preservation, along with the ability to produce functional foods for human and animal disease management (Shetty and Wahlqvist, 2004; Sarkar and Shetty, 2014).

The most significant biological functions of phenolics are the antioxidant properties that counter oxidative stresses (Bors and Michell, 2002; Close and Mc Arthur, 2002). Structurally, phenolic compounds are designed with an aromatic ring bearing one or more hydroxyl substitutes, including their functional derivatives (Rice-Evans *et al*., 1995). Phenolics can either scavenge free radicals directly or stimulate host antioxidant enzyme responses to counter oxidative stresses (Shetty and Wahlqvist, 2004). Diversity and heterogeneity of phenolic compounds in plants provide a broad spectrum of protective defenses against wide arrays of pathogens and pests (Nicholson and Hammerschmidt, 1992).

Beyond shielding and protecting against biotic stresses, phenolics can also potentially protect plant cells and tissues against oxidative damages induced by abiotic stresses, such as heat, cold, salinity, UV-B radiation, and wounding. The increased biosynthesis of phenolic compounds in plants increases abiotic stress resilience by enhancing antioxidative potentials and simultaneously improving the nutritional quality of food crops (Sarkar *et al*., 2010; 2011; Shetty and McCue, 2003). The

advantage of high antioxidant potential of plant phenolics promotes plant resilience against biotic and abiotic stresses, and can protect human health applications.

Plant-based foods are generally an excellent source of phenolic bioactives with significant antioxidant capacity. Thus, plant-based foods rich in phenolic bioactives and high antioxidant profile with potential to be successfully utilized in the dietary strategies for management of non-communicable diseases, such as type 2 diabetes and cardiovascular diseases (Dembinska-Kiec *et al*., 2008). The most common mechanism involved in the NCDs pathophysiology, including type 2 diabetes, is the existence of a chronic state of oxidative stress and subsequent breakdown of cellular equilibrium (Voehringer, 1999). Such chronic oxidative stresses can be countered by consuming plant-based foods with high levels of dietary antioxidants that offer safe, cost–effective prevention and cellular homeostasis through alternatives to pharmaceutical drugs, especially during early stages of the major NCDs.

In addition to antioxidant potentials, plant phenolics also possess antimutagenic, anticarcinogenic, and antiglycemic properties. Animal cells primarily respond to phenolic compounds through direct interactions with receptors or enzymes involved in the metabolic processes of digestion and signal transduction, or through modifying gene expression that can result in triggering a series of redox dependent reactions (Ordovas, 2006).

Phenolic bioactives influence glucose metabolism by inhibiting carbohydrate digestion and glucose absorption in the intestine, stimulating insulin secretion from pancreatic β-cells, modulating glucose release from liver, activating insulin receptors and glucose uptake in insulin-sensitive tissues, and modulating hepatic glucose output (Hanhineva *et al*., 2010). For effective management in the early stages of type 2 diabetes, carbohydrate digestion and glucose absorption are obvious targets for providing better glycemic control after consumption of hyper-processed, calorie-dense carbohydrate meals. The key enzymes for the digestion of dietary carbohydrates, α-amylase and α-glucosidase, are responsible for glucose absorbed in the small intestine. Inhibition of the digestive enzymes could slow the rate of glucose release and absorption in the small intestine, consequently suppressing postprandial hyper-glycemia (Hanhineva *et al*., 2010). Previous *in vitro* studies have reported that phenolic metabolites, phenolic acids, and tannins from different plant-based foods can significantly inhibit α-amylase and α-glucosidase activities (Cheplick *et al*., 2007; 2010; Fujita *et al*., 2015; Kwon *et al*., 2008, McCue *et al*., 2005; Pinto *et al*., 2008; Sarkar *et al*. 2015). Thus, plant-based foods with high α-amylase and α-glucosidase inhibitory activities are good choices for incorporating in the dietary strategies to manage hyperglycemia-linked early stages type 2 diabetes.

Resveratrol and other phenolics from red wines and grapes also have diverse biological functions in protecting human health through antioxidant capacity that provides protection against endothelial dysfunction, improves lipid metabolism, and has anticarcinogenic properties (Nigdikar *et al*., 1998; Soleas *et al*., 2002; Vitaglione *et al*., 2005). A previous *in vitro* study showed significant α-glucosidase inhibitions and moderate α-amylase inhibitions in several red wines (Kwon *et al*., 2008). More than improving glucose metabolism, red wine phenolics also have high antioxidant potentials that can provide additional protection against chronic oxidative stress that causes cellular breakdown in eyes and kidneys due to peripheral artery disease and neuropathy from microvascular complications experienced by type 2 diabetic patients.

The demand to develop novel strategies for designing functional foods and ingredients with diverse human health benefits is increasing worldwide. (Bagchi, *et al*., 2016). Enhancing bio-synthesis of phenolic bioactives in plant-based foods could potentially help prevent and manage NCDs. Available metabolic innovations may improve the biosynthesis of phenolic metabolites by controlling and modifying plant endogenous defense responses. Such innovation can frequently provide affordable alternatives and complimentary drug treatments in many countries. Mild induction of abiotic stresses, such as instantaneous heat shock can increase phenolic bio-synthesis and associated antioxidant enzyme activities in plant-based foods during pre- and post-harvest stages (Cisneros-Zevallos, 2003). High phenolic content and reduced peroxidase activity have been observed in heat shock treated carrots (Alegria *et al*., 2012). Similarly, browning was reduced after wounding in heat shocked lettuce leaf (Loaiza-Velarde and Saltveit, 2001). Thus, utilizing mild abiotic stress treatments that can improve phenolic bioactive profiles and antioxidant capacity in plant-based foods has significant merit and can be employed in designing functional foods with higher human health benefits. The main objective of this study was to evaluate the impact of instantaneous,

pre-harvest, heat shock treatments of grapes on red wines after fermentation.

MATERIALS AND METHODS

Heat shock treatment. Field treatments with Pinot Noir grape were tested at Adelsheim vineyard, Willamette Valley, Oregon (Latitude 45.371273, Longitude 123.069008) for three years (2012, 2013, 2014). In the year 2014, Pinot Noir grapes were heat-treated immediately after bud formation and then every ten days until bloom followed by treatment every four to five days during bloom and every 10 to 12 days until two to three weeks before veraison. The heat treatments were at 140º C using a moving thermal system (Agrothermal System, Walnut Creek, CA) using propane fuel-driven heat treatments directed at the grape vines and moving 3.5-4 miles per hour. In 2013, the Pinot Noir grape plants were treated with a 140°C treatment using the Agrothermal system at four miles per hour, with the grapes treated every five days during bloom and then every 10-12 days until two-three weeks before veraison. For the year 2012, grapes were treated, at 140° C, 3.5-4 miles per hour, starting at bloom every five days and after bloom every ten days until veraison. Controls followed conventional practices without the instantaneous heat shock treatments.

Sample preparation. Pinot Noir red wine was prepared each year by Adelsheim Vineyard following their standard protocol. Wine samples, three, from the 2014 batch and one sample each from the 2012 and 2013 batches were evaluated for this study. The samples were transported from Adelsheim Vineyard to the North Dakota State University in early 2015 and kept for one week under normal room temperature for post transportation stabilization. Wine samples were collected separately from the bottom, middle, and top layer using a pipette and then centrifuged for 15 minutes at 4038g. After centrifugation, wine samples were transferred to tubes and the pH of the samples were measured. Different biochemical and *in vitro* assays were done with and without adjusting pH of samples. The *in vitro* wine sample assays were kept under refrigeration for a maximum of one week. Fresh batches were used for repetitions of the experiment. The samples were received as blind samples with number coding and decoded only after complete biochemical analysis.

Total soluble phenolic assay. Total phenolic content of red wine sample was determined using an assay modified by Shetty *et al*. (1995). One milliliter of sample after two times dilution was transferred into test tube and added with 1 mL of 95% ethanol, 5 mL of distilled water and 0.5 mL of 50% (v/v) Folin-Ciocalteau reagent, respectively. The mixture was left to incubate for 5 min, followed by the addition of 1 mL of 5% Na_2CO_3. After thorough mixing, the reaction mixture was incubated in the dark for 60 min and the absorbance was read at 725 nm. Standard curves were generated using increasing concentrations of gallic acid in 95% ethanol. Absorbance values were converted to total phenolics and expressed as mg of gallic acid per mL volume of the red wine.

Antioxidant Activity by 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical inhibition assay. Antioxidant activity of red wine sample was measured using a modified DPPH radical inhibition assay (Cervato *et al*., 2000). In a micro centrifuge tube, a volume of 0.25 mL of a sample mixture was added to 1.25 mL of 60 µM DPPH in 95% ethanol. During 5 min of incubation, the samples were vortexed and then centrifuged at 15,000g for 1 min. The absorbance (A) was read at 517 nm. As a control, 0.25 mL of 95% ethanol was used instead of a sample mixture. The antioxidant activity was expressed as % inhibition of DPPH radical formation and calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(A_{517} \text{ control} - A_{517} \text{ sample})}{A_{517} \text{ control}} * 100$$

Alpha-glucosidase inhibition assay. The α-glucosidase inhibition assay of red wine samples were done by mixing 50 µL of red wine sample and 100 µL of 0.1 M phosphate buffer containing (pH 6.9) containing α-glucosidase enzyme solution (1.0 U/mL) in 96-well plates (Kwon *et al*., 2008). The mixture solutions were incubated at 25 °C for 10 min. After pre-incubation, 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 incubated at 25 °C for 5 min. Before and after the 5 min incubation, absorbance (A) reading were recorded at 405 nm by microplate reader (Thermomax, Molecular Device Co., VA, USA) and the difference between 0 and 5 min readings were noted as a change in absorbance. For the control, 50 µL of buffer solution was added instead of sample. The result was expressed as % inhibition of α-glucosidase and calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(\Delta A_{405} \text{ control} - \Delta A_{405} \text{ sample})}{\Delta A_{405} \text{ control}} * 100$$

Alpha-amylase inhibition assay. A total volume of 500 µL of red wine sample and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase enzyme solution (0.5 mg/mL) were incubated at 25 °C for 10 min (Kwon *et al*., 2008). After pre-incubation, 500 µL of 1% (w/v) starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at timed intervals and then incubated at 25 °C for 10 min. The reaction was stopped with the addition of 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were incubated in a boiling water bath for 10 min and then cooled to room temperature. The reaction mixture was subsequently diluted with 10 mL of distilled water and the absorbance (A) was read at 540 nm. Dose responses were determined by diluting red wine samples for two and five times. The result was expressed as % inhibition of α-amylase and calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(A_{540} \text{ control} - A_{540} \text{ sample})}{A_{540} \text{ control}} \times 100$$

Angiotensin-I-converting enzyme (ACE) inhibition assay. The ACE inhibition activity was measured using a modified (Cushman and Cheung, 1971; Kwon *et al*., 2008) method. A volume of 50 µL of red wine sample was incubated with 200 µL of 0.1 M NaCl-borate buffer (pH 8.3) containing 2.0 mmol ACE solution at 25 °C for 10 min. After pre-incubation, 100 µL of 5 mM substrate solution hippuryl-histidine-leucine (HHL) was added. The reaction mixture was incubated at 37 °C for one hour. The reaction was subsequently stopped with the addition of 150 µL of 0.5 N HCl. The product of ACE reaction, hippuric acid, was detected and quantified using HPLC.

A 5mL sample of the wine, using an Agilent ALS 1100, was injected into an Agilent 1200 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with an autosampler and a DAD 1100 diode array detector (Kwon *et al.*, 2008). The solvents used for gradient elution were 10 mM phosphoric acid (pH 2.5) and 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for the next 5 min, then decreased to 0% for the last 5 min (total run time is 18 min). The analytical column used was Nucleosil 100-5C18, 250x4.6 mm i.d., with packing material of 5 µm particle size at a flow rate 1 mL/min at ambient temperature. During each run, the chromatogram was recorded at 228 nm and integrated using Agilent Chemstation enhanced integrator for detection of liberated hippuric acid. The peak area of hippuric acid (E) chromatogram was noted. Pure hippuric acid was used to calibrate the standard curve and retention time. The result was expressed as % inhibition of ACE and calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{E control-E sample}}{\text{E control-E blank}} * 100$$

HPLC analysis of phenolic profiles. The red wine sample (2 mL) was filtered through a 0.2 µm filter. A volume of 5 µL sample was injected using the previously described Agilent 1200 series HPLC equipped with an auto-sampler and a DAD 1100 diode array detector. The solvents used for gradient elution were 10 mM phosphoric acid (pH 2.5) and 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% over the next 7 min, then decreased to 0% for the next 3 min and was maintained for the next 7 min (total run time is 25 min). The analytical column used was Agilent Zorbax SB-C18, 250x4.6 mm i.d., with packing material of 5 µm particle size at a flow rate of 1 mL/min at ambient temperature. During each run, the chromatogram was recorded at 225 nm and 306 nm and integrated using Agilent Chemstation enhanced integrator. Pure standards of gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, resveratrol, rutin, p-Coumaric acid, m-coumaric acid and rosmarinic acid in 100% methanol were used to calibrate the standard curves and retention times.

Statistical analysis. All experiments were replicated three times and *in vitro* analysis from each experiment was done in triplicates. Means and standard errors were calculated from the replicates within the experiments and analyzed using Microsoft Excel XP. Significant differences were determined using one way ANOVA, and a least significant difference test at $p < 0.05$.

RESULTS

Total soluble phenolics. Overall an increase in total soluble phenolic content in red wines from instantaneous heat shock treated Pinot Noir grapes was observed when compared with their respective controls (Figure 1). Only one batch of red wine from the year 2014 did not show any increment in total soluble phenolic content. Total soluble phenolic content of studied red wine samples varied between 1.01 to 1.33 mg/mL GAE. Among different sample years, the 2013

Pinot Noir red wine sample had highest total soluble phenolic content (1.31 and 1.33 mg/mL GAE) followed by the 2012 and 2014 batches. All three batches from the 2014 Pinot Noir red wine had lower total soluble phenolic content when compared with red wine batches from other years.

Antioxidant activity by DPPH radical inhibition assay. In all the studied red wine samples, antioxidant activity was improved in wines made from instantaneous heat shock treated grapes when compared with their respective controls (Figure 2). The improvement in antioxidant activity of red wine samples ranged between 2-4%. Interestingly, total antioxidant activity did not correlate positively with total soluble phenolic content in this study. Red wine samples from all three of the 2014 batches had higher total antioxidant activity (68-78% DPPH inhibitions) followed by 2013 and 2012 red wine samples, however, some red wine from the 2014 batch had lower total soluble phenolic content.

Figure 1. Total soluble phenolic content of red wines derived from heat-shock treated and untreated controls. The bar shows standard error associated with each treatment.

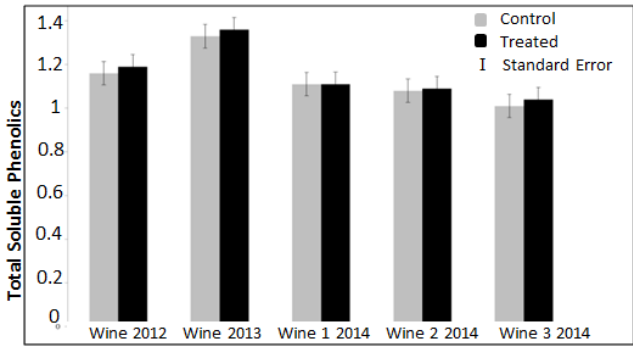
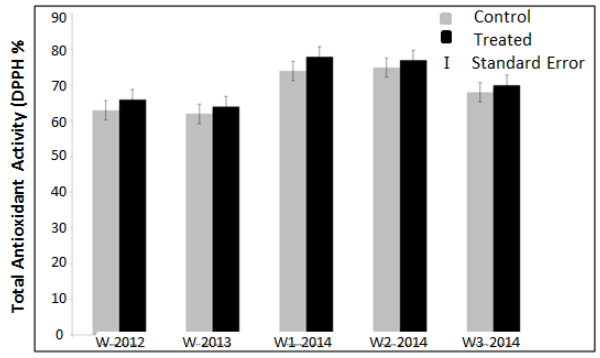


Figure 2. Total antioxidant activity of wines derived from heat-shock treated and untreated controls; with (treated) and without (control) instantaneous heat shock treated Pinot Noir grapes. The bar shows standard error associated with each treatment.



Alpha-glucosidase inhibition assay. In the current study, a high α-glucosidase inhibition (94-100%) was observed in undiluted samples of red wines derived from both control and instantaneous heat shock treated Pinot Noir grapes (Figure 3). No statistically significant differences, however, were observed between control and heat shock treated grape derived red wine samples, although the trend line towards stimulation in response to heat stress was noted. Not finding significant differences in these red wines was probably due to very high α-glucosidase inhibitory activity (78-84%) even after 1:5 dilution. Thus, due to higher α-glucosidase inhibitions in control, a saturation effect and no scopes for further observable significant improvement with instantaneous heat shock treatments of the grape were observed, however red wine can be targeted to manage chronic hyperglycemia.

Alpha-amylase inhibition assay. Another important enzyme involved in human glucose metabolism is α-amylase and can be targeted for lowering post prandial blood glucose level. Alpha-amylase inhibition was significantly improved in red wines derived from instantaneous heat shock treated grapes when compared to their respective controls (Figure 4). The improvements were more prominent in higher diluted samples (after 1/5th dilution) as undiluted samples showed very high (90-95%) α-amylase inhibition. Significant dose responses in α-amylase inhibitions were observed in all red wine samples. Such results indicated that even after dilution red wines derived from instantaneous heat shock treated grapes hold moderate to high α-amylase inhibition.

ACE inhibition assay. One of the most important intermediary factors for controlling hypertension is the action of the angiotensin I-converting enzyme (ACE-I) (Hernandez-Ledesma *et al*., 2005). Inhibition of ACE-I is considered a beneficial therapeutic approach in the treatment of high blood pressure in both diabetic and non-diabetic patients (Erdos and Skidgel, 1985). In this study, no inhibitory activity of ACE-I was observed in red wine samples derived from instantaneous heat shock treated grapes.

HPLC phenolic profiles. Different soluble phenolic compounds of red wine samples were determined through HPLC analysis. Major phenolic compounds found in studied red wine samples were caffeic acid, gallic acid, p-coumaric acid, and quercetin. Concentration of caffeic acid (33-45 µg/mL) and gallic acid (42-48 µg/mL) were significantly higher in studied red wine samples compared to the other two phenolics (p-coumaric 15-21 µg/mL and quercetin 18-24 µg/mL) (Table 1). Similar phenolic profiles were reported in other red wine samples previously (Kwon et al., 2008).

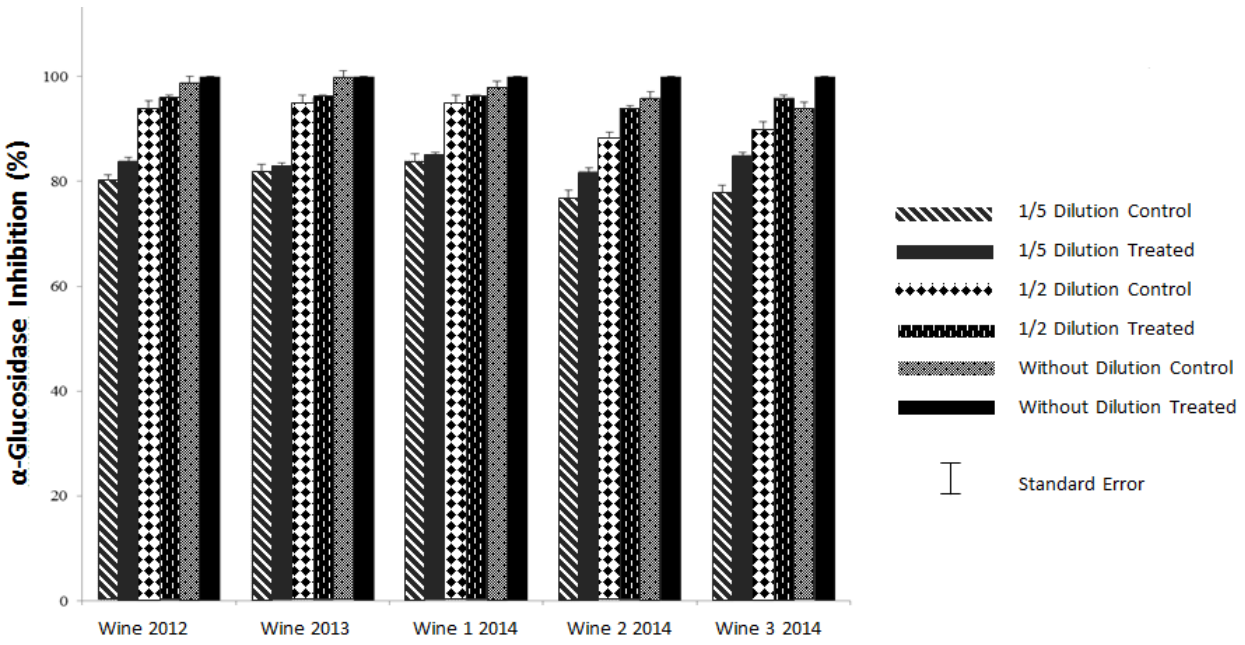


Figure 3. α-glucosidase inhibition of red wines derived from heat-shock treated and untreated controls. The bar shows standard error associated with each treatment.

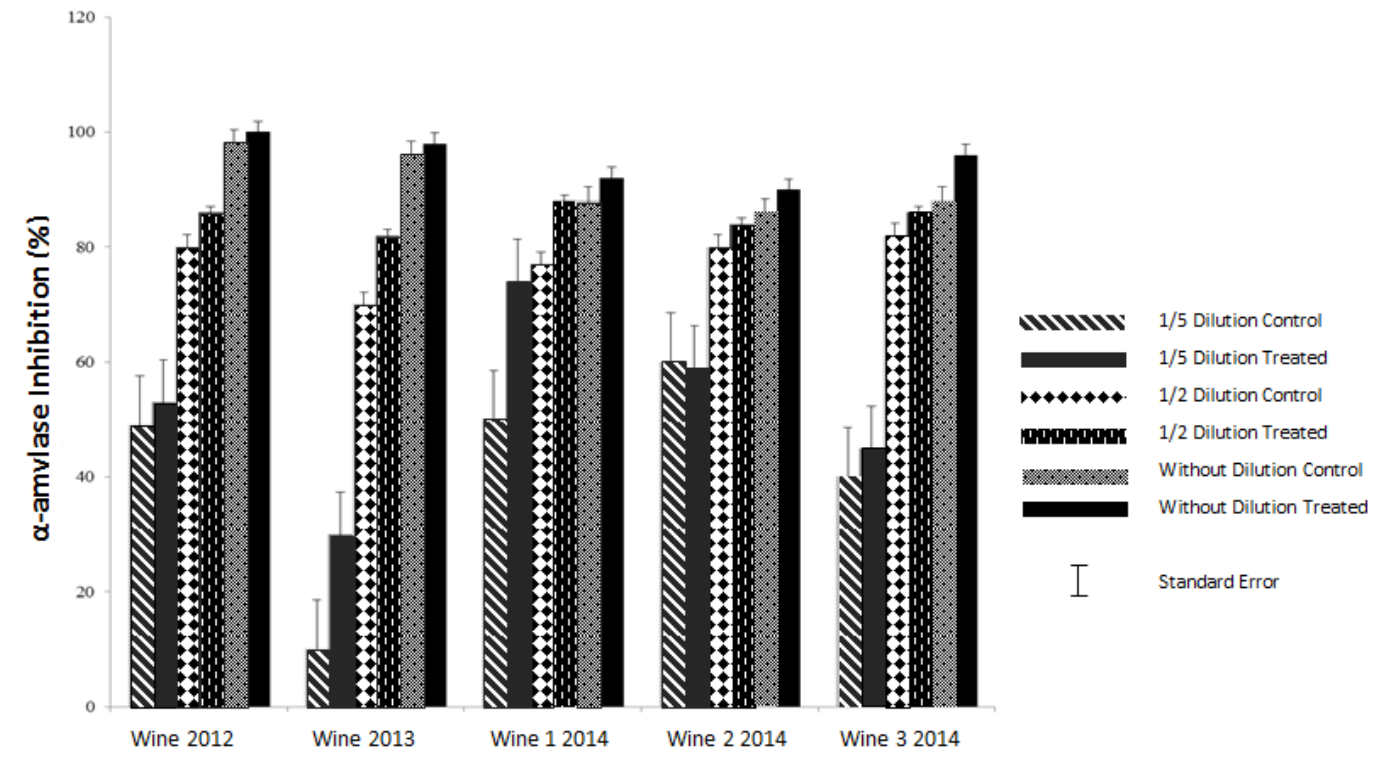


Figure 4. α-glucosidase inhibition of red wines derived from heat-shock treated and untreated controls. The bar shows standard error associated with each treatment.

Table 1. Phenolic profile of red wines derived from controls and instantaneous heat shock treated Pinot Noir grapes.

Phenolics	Wine 2012		Wine 2013		Wine1 2014		Wine 2 2014		Wine 3 2014	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Caffeic acid	39.04	40.07	41.02	45.02	36.08	35.07	33.08	35.00	33.04	36.05
Gallic acid	44.26	47.24	45.03	48.06	42.04	44.23	43.66	47.52	42.05	46.05
p-Coumaric acid	16.45	19.23	18.07	21.02	16.72	17.23	15.24	16.82	15.07	15.04
Quercetin	22.23	24.02	20.24	24.23	20.13	21.22	19.04	20.24	18.45	20.42

DISCUSSION

Phenolic antioxidant-linked anti-hyperglycemic and anti-hypertensive properties of Pinot Noir red wines derived from instantaneous heat shock treated and untreated grapes were investigated. Significant variations in total soluble phenolic content among different batches of red wines from different years were observed in this study. Differences in instantaneous heat shock treatments (temperature and timing), environmental factors, and other growing conditions across different years may have resulted in the variations of total soluble phenolic content in different batches of red wines.

Higher total soluble phenolic content in red wine from instantaneous heat shock treated Pinot Noir grape potentially indicate the pre-harvest stimulation of phenolic bio-synthesis due to abiotic stress induction. Further analyzation of this is being performed under a separate fruit study. Similar post-harvest stimulation of total phenolics was observed in carrots and in iceberg lettuce (Algeria *et al.,* 2012; Loaiza-Velarde and Saltveit, 2001). Such incremental increase of total soluble phenolic content in red wine translating from instantaneous heat shock treated grape has different implications including potential improvement of food and nutritional qualities, extension of shelf life and better preservation, and significance for human health relevant applications.

Potential pre-harvest stimulation of phenolic bio-synthesis may also provide protection to grape plants against biotic and abiotic stresses. Among phenolic acid profile, instantaneous heat shock treatment only increased gallic acid concentrations in derived red wine samples. Other phenolics in red wine samples did not change significantly with instantaneous heat shock treatments of source fruits. Improvement of gallic acid concentrations in red wine derived from instantaneous heat shock treatment of Pinot Noir grape has significant human health and food preservation relevant implications and can be utilized for designing new functional food ingredients (Kaur *et al.,* 2009; Lu *et al.,* 2006).

High total antioxidant activity was also observed in Pinot Noir red wines derived from instantaneous heat shock treated and untreated grape sample. No positive correlations between total soluble phenolic content and total antioxidant activity, however, were observed in this current study. A similar level of total antioxidant activity in red wine samples was also previously reported by Kwon *et al.* (2008). Increase in total antioxidant activity in red wine from instantaneous heat shock treated grape potentially indicates stimulation of enzymatic as well as non-enzymatic antioxidant responses after heat stress induction. Such antioxidant stimulation may be independent to the bio-synthesis of phenolic metabolites in these grapes. Antioxidant potential of different red wine phenolics may vary based on the phenolic compositions and fractions (Ghiselli *et al.,* 1998). Mild antioxidant stimulation in red wine from heat shock treated grapes has diverse benefits, especially to counter oxidative stress induced damages in both plant tissues at pre-harvest and post-harvest stages as well as in human cells when wine is consumed as a beverage. Thus, instantaneous heat stress can be used to enhance medicinal bioactives to combat oxidative stress-linked NCDs including management of early stages of type 2 diabetes.

Maintaining redox as well as glucose homeostasis is critical to manage chronic hyperglycemia and oxidative stress commonly associated with type 2 diabetes. Alpha-glucosidase is an important enzyme that helps break starch and disaccharides into glucose and facilitates subsequent absorption in the small intestine (Lebovitz 1997). Many anti-diabetic pharmaceutical drugs such as Acarbose have been designed to inhibit Alpha-glucosidase for slowing down the breakdown and absorption of glucose in the small intestine. Thus, such mechanisms provide excellent strategy to reduce the postprandial blood glucose rise both in diabetic and pre-diabetic patients. Phenolics from plant-based foods possess significant α-glucosidase inhibitory activities and are safer than pharmaceutical drugs (Kim *et al.,* 2000). In the current study, very high α-glucosidase

inhibitory activity and dose responses were previously reported in four different red wine samples (Kwon *et al.* 2008). Overall, high α-glucosidase inhibitory activity in this study showed significant potential for incorporating red wines in dietary strategies to combat hyperglycemia-linked early stages of type 2 diabetes.

Similar to α-glucosidase inhibition, plant phenolics from different plant based foods have also shown significant α-amylase inhibitory activities in previous *in vitro* as well as in *in vivo* studies (Kwon *et al.*, 2008; McCue *et al.*, 2005; Tadera *et al*., 2006). Previous *in vitro* studies with red wine reported comparatively lower α-amylase inhibitory activities (Kwon *et al.*, 2008). Such variations in α-amylase inhibitions may be due to the differences of grape cultivars used to make these red wines along with differences in the growing conditions.

Both genotypic and phenotypic variations significantly affect phenolic and antioxidant profiles in plant-based foods and the subsequent potential to inhibit enzymes relevant to human glucose metabolism. In this study, α-amylase inhibitory activities of red wine samples positively correlated with the total phenolic content. The result has important implications as instantaneous heat shock treatments have significant potential for improving phenolic antioxidant-linked α-amylase inhibitions in plant-based foods and derived beverages for dietary management of the early stages of type 2 diabetes.

Similar to hyperglycemia, hypertension is also a common risk factor associated with type 2 diabetes and cardiovascular diseases. Therefore inhib-itory potential of Pino Noir red wine against key hypertension-linked enzyme (ACE-I) was also investigated in this study. No ACE inhibitory activity in red wines, however, was derived from instantaneous heat shock treated and untreated grapes.

Previous *in vitro* studies with red wines also showed no ACE inhibitory activities (Kwon *et al.*, 2008). Only purified peptide fractions from Muscat Bailey red wine showed ACE-I inhibitory properties (Takayanagi and Tokotsuka, 1999). Similarly, red wine vinegar beverages exhibited ACE-I dependent inhibitions in *in vivo* study (Honsho *et al.*, 2005). Thus, red wines in original state may not show any ACE-I inhibitory activity, but with further processing such anti-hypertension properties can be potentially induced. The role of red wine for improve coronary heart disease may not be associated with ACE-I inhibitions but due to their other human health relevant benefits such as blocking human platelet aggregations, inhibitions of oxidation of low density lipoprotein (LDL), and inhibition of synthesis of vasoactive peptide (Corder *et al.*, 2001; Frankel *et al.*, 1993; Pace-Asciak *et al.*, 1995).

CONCLUSION

The present study provides significant insights about the potential of using instantaneous abiotic heat stress in grape during pre-harvest stages for enhancing phenolic bioactive-linked medicinal properties in derived wines. Further such improvement has significant relevance in designing dietary strategies with phenolic bioactive enriched foods and beverages to counter diet and oxidative stress-induced NCDs, such as management of early stages type 2 diabetes. Due to rich medicinal properties, grape and grape derived beverages (wines) have traditionally been used as medicinal foods to counter disease and manage human health. The present study confirmed the medicinal values of red wine especially the potential of red wine to inhibit major enzymes involved in human glucose metabolism such as α-amylase and α-glucosidase in *in vitro* assays. Research findings of this study showed that instantaneous heat shock treatments in Pinot Noir grapes during pre-harvest stages significantly improved total phenolic content, total antioxidant activity, and α–amylase inhibitory activity in red wines derived from heat stressed grapes. Though no statistically significant observable differences between control and heat shock derived grape wine for α-glucosidase inhibitory activity was found, a positive stimulatory trend was evidenced. The findings have significant implications for understanding the positive beneficial impact of heat-shock treatment during fruit pre-harvest stages in improving medicinal properties of derived wines. Utilizing such innovative strategies to design new functional food products and ingredients to manage the global epidemic of type 2 diabetes and other NCDs is very promising.

REFERENCES

Alegria, C., J. Pinheiro, M. Duthoit, E.M. Gonçalves, M. Moldão-Martins, and M. Abreu. 2012. Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress (heat shock and UV-C irradiation) pre-treatments. LWT-Food Science and Technology 48: 197-203.

Bagchi, D. and S. Nair. 2016. Developing New Functional Food and Nutraceutical Products. Elsevier Inc.

Bors, W. and C. Michel. 2002. Chemistry of the antioxidant effect of polyphenols. Ann. N.Y. Acad. Sci. 957: 57-69.

Cervato, C., M. Carabelli, S. Gervasio, A. Cittera, R. Cazzola, and B. Cestaro. 2000. Antioxidant properties of oregano (*Origanum vulgare)* leaf extracts. J. Food Biochem. 24: 453-465.

Cheplick, S., Y-I. Kwon, P.C. Bhowmik, and K. Shetty. 2010. Phenolic-linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension. Bioresource Technology 101: 404-413.

Cheplick, S., Y-I. Kwon, P.C. Bhowmik, and K. Shetty. 2007. Clonal variation in raspberry fruit phenolics and relevance for diabetes and hypertension management. Journal of Food Biochemistry 31: 656-679.

Cisneros-Zevallos, L. 2003. The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables. Journal of Food Science 68: 1560-1565.

Close, D.C. and C. McArthur. 2002. Rethinking the role of many plant phenolics- protection from photodamage not herbivores? Oikos 99: 166-172.

Corder, R., J.A. Douthwaite, D.M. Lees, N.Q. Khan, A.C.V. dos Santos, E.G. Wood, and M.J.Carrier. 2001. Health: Endothelin-1 synthesis reduced by red wine. Nature 414: 863-864.

Cushman, D.W. and H.S. Cheung. 1971. Spectrophotometric assay and properties of angiotensin converting enzme of rabbit lung. Biochem Pharmacol. 20: 1637-1648.

Dembinska-Kiec, A., O. Mykkänen, B. Kiec-Wilk, and H. Mykkänen. 2008. Antioxidant Phytochemicals against type 2 diabetes. British Journal of Nutrition 99: ES109-ES117.

Erdos, E.G. and R.A. Skidgel. 1985. Structure and functions of human angiotensin I converting enzyme (kininase II). Biochemical Society Transactions 13: 42-44.

Frankel, E.N., J.B. German, J.E. Kinsella, E. Parks, and J. Kanner. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. The Lancet 341: 454-457.

Fujita, A., D. Sarkar, S. Wu, E. Kennelly, K. Shetty, and M.I. Genovese. 2015. Evaluation of phenolic-linked bioactives of camu-camu (*Myrciaria dubia* Mc. Vaugh) for antihyperglycemia, antihypertension, antimicrobial properties and cellular rejuvenation. Food Research International 77: 194-203.

Ghiselli, A., M. Nardini, A. Baldi, and C. Scaccini. 1998. Antioxidant activity of different phenolic fractions separated from an Italian red wine. Journal of Agricultural and Food Chemistry 46: 361-367.

Hanhineva, K., R. Törrönen, I. Bondia-Pons, J. Pekkinen, M. Kolehmaine, H. Mykkänen, and K. Poutanen. 2010. Impact of dietary polyphenols on carbohydrate metabolism. International Journal of Molecular Sciences 11: 1365-1402.

Hernández-Ledesma, B., B. Miralles, L. Amigo, M. Ramos, and I. Recio. 2005. Identification of antioxidant and ACE-inhibitory peptides in fermented milk. Journal of the Science of Food and Agriculture 85: 1041-1048.

Honsho, S., A. Sugiyama, A. Takahara, Y. Satoh, Y. Nakamura, and K. Hashimoto. 2005. A red wine vinegar beverage can inhibit the renin-angiotensin system: experimental evidence in vivo. Biological and Pharmaceutical Bulletin 28: 1208-1210.

Kaur, M., B. Velmurugan, S. Rajamanickam, R. Agarwal, and C. Agarwal. 2009. Gallic acid, an active constituent of grape seed extract, exhibits anti-proliferative, pro-apoptotic and anti-tumorigenic effects against prostate carcinoma xenograft growth in nude mice. Pharmaceutical research 26: 2133-2140.

Kim, J.S., C.S. Kwon, and K.H. Son. 2000. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Bioscience, biotechnology, and biochemistry 64: 2458-2461.

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

Lebovitz, H.E. 1997. Alpha-glucosidase inhibitors. Endocrinology and metabolism clinics of North America 26: 539-551.

Loaiza-Velarde, J.G. and M.E. Saltveit. 2001. Heat shocks applied either before or after wounding

reduce browning of lettuce leaf tissue. Journal of the American Society for Horticultural Science 126: 227-234.

Lu, Z., G. Nie, P.S. Belton, H. Tang, and B. Zhao. 2006. Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. Neurochemistry international 48: 263-274.

McCue, P., Y-I. Kwon, and K. Shetty. 2005. Anti-amylase, anti-glucosidase and anti-angiotensin I- converting enzyme potential of selected foods. Journal of Food Biochemistry 29: 278-294.

Nicholson, R.L. and R. Hammerschmidt. 1992. Phenolic compounds and their role in disease resistance. Ann. Rev. Phytopathol. 30: 369-389.

Nigdikar, S.V., N.R. Williams, B.A. Griffin, and A.N. Howard. 1998. Consumption of red wine polyphenols reduces the susceptibility of low-density lipoproteins to oxidation *in vivo*. The American Journal of Clinical Nutrition 68: 258-265.

Ordovas, J.M. 2006. Genetic interactions with diet influence the risk of cardiovascular disease. Am. J. Clin. Nutr. 83: 443S-446S.

Pace-Asciak, C.R., S. Hahn, E.P. Diamandis, G. Soleas, and D.M. Goldberg. 1995. The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. Clinica. Chimica. Acta. 235: 207-219.

Pinto, M., Y-I. Kwon, E. Apostolidis, F.M. Lajolo, M.I. Genovese, and K. Shetty. 2008. Functionality of bioactive compounds in Barzillian strawberry *(Fragaria x ananassa* Duch.) cultivars: Evaluation of hyperglycemia and hypertension potential using *in vitro* models. J. Agric. Food Chem. 56: 4386-4392.

Rice-Evans, C.A., N.J. Miller, P.G. Bolwell, P.M. Bramley, and J.B. Pridhan. 1995. The relative antioxidant activities of plant derived polyphenolic flavonoids. Free Radic. Biol. Med. 22: 375-383.

Sarkar, D. and K. Shetty. 2014. Metabolic Stimulation of Plant Phenolics for Food Preservation and Health. Annual Review of Food Science and Technology 5: 395-413.

Sarkar, D., C. Ankolekar, M. Pinto, and K. Shetty. 2015. Dietary functional Benefits of Bartlett and Starkrimson pears for potential management of hyperglycemia, hypertension and ulcer bacteria Helicobacter pylori while supporting beneficial probiotic bacterial response. Food Research International 69: 80-90.

Sarkar, D., P.C. Bhowmik, and K. Shetty. 2010. Effects of marine peptide and chitosan oligosaccharide on high and low phenolic creeping bentgrass clonal lines and improvement of antioxidant enzyme response. Agronomy Journal 102: 981-989.

Sarkar, D., P.C. Bhowmik, Y-I. Kwon, and K. Shetty. 2011. The role of proline-associated pentose phosphate pathway in cool-season turfgrasses after UV-B exposure. Environmental and Experimental Botany 70: 251-258.

Shetty, K. and P. McCue. 2003. Phenolic antioxidant biosynthesis in plants for functional food application: Integration of system biology and biotechnological approaches. Food Biotechnology 17:67-97.

Shetty, K. and M.L. Wahlqvist. 2004. A model for the role of proline-linked pentose phosphate pathway in phenolic phytochemical biosynthesis and mechanism of action for human health and environmental applications; A Review. Asia Pacific J. Clinical Nutrition 13: 1-24.

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

Soleas, G.J., L. Grass, P.D. Josephy, D.M. Goldberg, and E.P. Diamandis. 2002. A comparison of the anticarcinogenic properties of four red wine polyphenols. Clinical Biochemistry 35: 119-124.

Tadera, K., Y. Minami, K. Takamatsu, and T. Matsuoka. 2006. Inhibition of. ALPHA.-Glucosidase and. ALPHA.-Amylase by Flavonoids. Journal of nutritional science and vitaminology 52: 149-153.

Takayanagi, T. and K. Yokotsuka. 1999. Angiotensin I converting enzyme-inhibitory peptides from wine. American journal of enology and viticulture 50: 65-68.

Vitaglione, P., S. Sforza, G. Galaverna, C. Ghidini, N. Caporaso, P.P. Vescovi, V. Fogliano, and R. Marchelli. 2005. Bioavailability of trans-

resveratrol from red wine in humans. Molecular Nutrition & Food Research 49: 495-504.

Voehringer, D.W. 1999. BCL-2 and glutathione: alterations in cellular redox state that regulate apoptosis sensitivity. Free Rad. Biol. Med. 27: 945:950.