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# Application of muscadine grape (*Vitis rotundifolia* Michx.) pomace extract to reduce carcinogenic acrylamide

Changmou Xu [a], Yavuz Yagiz [a], Sara Marshall [a], Zheng Li [a], Amarat Simonne [b], Jiang Lu [c], Maurice R. Marshall [a,*]

[a] Department of Food Science and Human Nutrition, University of Florida, Gainesville 32611, USA
[b] Department of Family, Youth and Community Sciences, University of Florida, Gainesville 32611, USA
[c] College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, People's Republic of China

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ABSTRACT

Acrylamide is a byproduct of the Maillard reaction and is formed in a variety of heat-treated commercial starchy foods. It is known to be toxic and potentially carcinogenic to humans. Muscadine grape polyphenols and standard phenolic compounds were examined on the reduction of acrylamide in an equimolar asparagine/glucose chemical model, a potato chip model, and a simulated physiological system. Polyphenols were found to significantly reduce acrylamide in the chemical model, with reduced rates higher than 90% at 100 μg/ml. In the potato chip model, grape polyphenols reduced the acrylamide level by 60.3% as concentration was increased to 0.1%. However, polyphenols exhibited no acrylamide reduction in the simulated physiological system. Results also indicated no significant correlation between the antioxidant activities of polyphenols and their acrylamide inhibition. This study demonstrated muscadine grape extract can mitigate acrylamide formation in the Maillard reaction, which provides a new value-added application for winery pomace waste.



## 1. Introduction

Acrylamide is a byproduct of the Maillard reaction and can be generated from food components, during heat treatment, as a result of the reaction between the reactive carbonyl group on the sugar and amino group of the amino acid (Mottram, Wedzicha, & Dodson, 2002). Acrylamide in food is a concern because it can cause cancer in laboratory animals at high doses, and is reasonably accepted to be a human carcinogen (NTP, 2011). In 2013, the FDA called attention to acrylamide in foods by publishing a draft guidance for industry, providing information to help growers, manufacturers, and food service operators on how to reduce the concentration of acrylamide in certain foods (FDA, 2013).

Since scientists announced the discovery of acrylamide formation in a variety of heated foods in 2002 (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002), many food additives have been studied for their inhibitory role on acrylamide formation. Some amino acids (Kim, Hwang, & Lee, 2005), sodium hydrocarbonate (Amrein, Schönbächler, Escher, & Amadò, 2004), and food antioxidants (Becalski, Lau, Lewis, & Seaman, 2003; Fernández, Kurppa, & Hyvönen, 2003) were found to be effective inhibitors against acrylamide formation. However, studies on the mechanism of action of potential inhibitors are ongoing and need to continue. This scavenging of free radicals and reactions with intermediates of acrylamide formation has been proposed for acrylamide reduction mechanisms using certain inhibitors (Cheng, Zeng, et al., 2009; Zeng et al., 2010). It was hypothesised that polyphenols may also reduce acrylamide formation, because of their strong antioxidant activity.

Muscadine grapes (*Vitis rotundifolia* Michx.) are native to the southeastern United States and were the first native grape species to be cultivated in North America (Andersen, Crocker, & Breman, 2010). There are over 100 improved cultivars of muscadine grapes. Most scientists divide the *Vitis* genus into two subgenera: *Euvitis* (the European, *Vitis vinifera* L. grapes and the American bunch grapes, *Vitis labrusca* L.) and the *Muscadinia* grapes (muscadine grapes) (Andersen et al., 2010). *Muscadinia* is instinctively different from the *Euvitis* since it has two more somatic chromosomes with numbers of 40 versus 38, respectively (Andersen et al., 2010). Muscadine grapes contain a large variety of antioxidant polyphenols (Xu, Yagiz, Borejsza-Wysocki, et al., 2014), and have several unique and distinguishing chemical compositions, particularly

* Corresponding author at: University of Florida, Food & Environmental Toxicology Lab, 1642 SW 23rd Dr., Bldg. 685, PO Box 110720, Gainesville, FL 32611-0720, USA. Tel.: +1 352 294 3981; fax: +1 352 392 1988.
*E-mail address:* martym@ufl.edu (M.R. Marshall).

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the presence of ellagic acid. Ellagic acid is commonly present in other fruits, such as raspberry, strawberry and blackberry, but is absent in all other *Vitis* species (Talcott & Lee, 2002). Also, unique to muscadine grapes is the presence of myricetin in the bronze grapes, as this flavonol is not present in white *V. vinifera* L. grapes (Flora, 1978). Another unique attribute of muscadine fruit chemistry is the presence of anthocyanins, such as 3,5-diglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, in non-acylated forms (Flora, 1978).

Dietary polyphenols have been proposed to be promising functional food additives due to their potent antioxidant capacity and other health-beneficial bioactivities (Li, Jiang, Xu, & Gu, 2015; Xu, Yagiz, Hsu, et al., 2014). As an example, winery pomace was reported to be a good source of polyphenols and dietary fibres, and could be incorporated into baked goods as a functional ingredient (Walker, Tseng, Cavender, Ross, & Zhao, 2014). While a recent study mentioned that the *V. vinifera* L. grape seed proanthocyanidins could mitigate acrylamide formation in an asparagine/glucose chemical model (Zhu, Cai, Ke, & Corke, 2009), other grape polyphenols, especially the unique muscadine grape (*V. rotundifolia* Michx.) polyphenols, are still an unknown. A comprehensive study of muscadine grape polyphenols on acrylamide reduction could develop a new potential application for the thousands of tons of winery pomace, which may be utilised as a functional food ingredient in a variety of heated starchy foods.

In this study, the effects of muscadine grape polyphenols, polyphenol fractions, and standard phenolic compounds were investigated on acrylamide reduction in an equimolar asparagine/glucose chemical model, a potato chip model, and a simulated physiological system. A correlation between antioxidant activities of phenolic compounds and their acrylamide inhibition, the influence of phenolic compounds on colour development in an acrylamide-producing chemical model, and the possible inhibition mechanism of acrylamide by polyphenols were also studied.

## 2. Materials and methods

### 2.1. Chemicals

Folin & Ciocalteu's phenol reagent (2 N), caffeic acid, gallic acid, catechin, epicatechin, epicatechin gallate, ellagic acid, quercetin, delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7, 8-tetramethyl-chroman-2-carboxylic acid (Trolox) were obtained from Sigma–Aldrich (St. Louis, MO). Acrylamide standard ($\geqslant$99.8%), L-asparagine, D-(+)-glucose, phosphate-buffered saline (PBS, pH 7.4) and all other chemicals and solvents were purchased from Fisher Scientific Co. (Pittsburgh, PA).

### 2.2. Grape materials

Fully ripened Muscadine grapes (10 varieties: cv. Alachua, cv. Carlos, cv. Doreen, cv. Fry, cv. Grany val, cv. Ison, cv. Majesty, cv. Noble, cv. Pam, cv. Supreme) were harvested from the Center for Viticulture and Small Fruit Research (latitude 30.65 N, longitude 84.60 W) at Florida A&M University in 2013. The collected samples were shipped to the University of Florida on the same day and stored in a cold room (4 °C). Grape skin and seeds were separated manually from berries and freeze-dried (Advantage, The Virtis Company, NY, USA). The freeze-dried samples were stored in vacuum-packaged polyethylene pouches at −20 °C until needed. A commercial table grape cv. Red Seed was used as a varietal control and purchased in 2013 from a local Wal-Mart Store in Gainesville, FL.

### 2.3. Sample preparation

Freeze-dried grape skin (20 g) was ground with a stainless-steel grinder (Omni-Mixer 17105, OCI Instruments, CT, USA) for 1 min, and then placed in a sieve ($\leqslant$0.25 mm). The fine powder passing through the sieve was collected (Xu, Zhang, Wang, & Lu, 2010). The powdered samples were stored at −20 °C and used for subsequent analysis.

Freeze-dried grape seeds (20 g) were crushed and then defatted with hexane at a ratio of 1:10 (w/v). After 24 h extraction at room temperature (shaking every 6 h), the hexane extract was filtered using Whatman #4 filter paper (0.45 μm) (Fisher Scientific, Pittsburgh, PA) under vacuum. The residue was evenly distributed over a tray and kept in the hood to evaporate the hexane. The final defatted grape seed powder was ground again in the stainless-steel grinder, and the powder passing through the sieve ($\leqslant$0.25 mm) was collected. The samples were also stored at −20 °C and used for subsequent analysis.

### 2.4. Phenolic compound extraction

Powder (0.5 g) from each sample was extracted with 10 ml of 70% methanol. The extraction flasks were vortexed for 30 s, sonicated for 10 min, kept at room temperature (22 °C) for 60 min, and sonicated for an additional 5 min. The extracts were transferred into tubes, centrifuged at 2820×*g*, 0 °C for 10 min (J-LITE® JLA-16.250, Beckman Coulter Inc., CA, USA), and the supernatant was collected in separate glass tubes. The residue was re-extracted by the same procedure. The pooled supernatant (20 ml) was filtered (0.45 μm) and used for further analysis.

### 2.5. Preparation of grape polyphenol fractions

Noble seed and skin polyphenol extracts were separated by HPLC and fractions were collected every 10 min, starting from the beginning of the run and terminated after 50 min. Five fractions were collected for each. To obtain a sufficient volume for the study, multiple separations of each extract were performed and then combined. The collected fractions were evaporated in a rotary evaporator, under reduced pressure at 40 °C, to remove solvent. The concentrates obtained after evaporation were dissolved in methanol and stored at 4 °C until analysed.

### 2.6. Phenolic compound analysis

The total phenolic content in grape seeds or skins was determined by the method of Singleton and Rossi (1965) using an ultraviolet–visible Beckman Coulter DU-640 spectrophotometer (Beckman Instruments, CA, USA). The properly diluted extract, 3900 μl of distilled water, 250 μl of Folin–Ciocalteau reagent (2 N), and 750 μl of 20% $Na_2CO_3$ were mixed in a tube. After reacting for 30 min in a 40 °C water bath, absorbance was measured at 760 nm. Gallic acid (GA) was used as a standard and expressed as gallic acid equivalents (mg gallic acid (GAE)/g dry matter (DM)) using a calibration curve. The linearity range of the calibration curve was 100–800 μg/ml ($R^2$ = 0.9996).

### 2.7. Antioxidant activity analysis

The DPPH assay was based on the method of Xu, Zhang, Cao, and Lu (2010). The properly diluted sample (100 μl) was added to 3.9 ml methanolic solution of DPPH (0.0025 g/100 ml $CH_3OH$). After 60 min reaction at room temperature in the dark, the absorbance at 515 nm was measured to determine the concentration of the remaining DPPH. The percent inhibition of DPPH in the test sample and known concentrations of Trolox were calculated by