Color and Polyphenolic Stability in Extracts Produced from Muscadine Grape (*Vitis rotundifolia*) Pomace

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The muscadine grape (*Vitis rotundifolia*) industry of the southern United States is largely devoid of value-added processes that capture the phytochemical content of wine and juice byproducts. Methods to recover and stabilize polyphenolics from muscadine grape pomace following juice manufacture were evaluated in laboratory-scale and pilot-scale trials. In laboratory-scale trials using osmotic equilibration, water-based extracts from juice pomace initially extracted 31−42% of total polyphenolics, 26−32% of total ellagic acid, and 36−62% of total anthocyanins. When adsorbed onto Amberlite XAD-4 resin to concentrate polyphenolics, these extracts lost 10.5% of their total ellagic acid from inefficient adsorption to the solid phase support. Subsequent pilot-scale trials were evaluated using hot water extracts from grape juice pomace followed by aerobic yeast fermentation to remove sugars and comparison to reversed phase C_{18} and Amberlite XAD-4. Extracts were also concentrated using spray-drying and vacuum evaporation. Fermentation had a minor impact on the retention of most polyphenolic compounds evaluated, yet resulted in a 16.3% decrease in antioxidant capacity. Spray-drying resulted in a 30.3% loss in total anthocyanins, a 21.5% loss in total phenolics, and a 23.3% decrease in antioxidant activity, whereas vacuum evaporation had no deleterious impact on these parameters. The physiology of the muscadine grape and its unique phytochemical composition has limited utilization of pomace from wine and juice manufacture. However, these studies demonstrated the potential to extract and concentrate polyphenolic-rich extracts for use in value-added applications.

KEYWORDS: Muscadine grape skins; pomace; polyphenolics; color stability; fermentation

INTRODUCTION

The ability of fruit and vegetable processors to utilize edible byproducts for the development of new products and revenue streams is a critical need in the food industry. The grape industry has long been a leader in these applications with products such as grape seed extract, dried pomace, and pigment extracts. However, the muscadine grape (*Vitis rotundifolia*) industry in the southeastern United States has lagged in the development of secondary products from wine and juice manufacture due to economic and processing limitations associated with this grape. Grown for their ability to withstand hot and humid summers and warm winters, this grape has a natural adaptation against Pierce's disease and is utilized commercially to manufacture wine, juice, and preserves (*1*, *2*). The grape is characterized by a thick, pectin-laden skin that retains appreciable amounts of antioxidant polyphenolics and simple sugars (*3*). The thick skins also cause difficulties during drying and are susceptible to browning, whereas less polar polyphenolics such as free ellagic acid are poorly solubilized during extraction procedures. The skins of muscadine grapes may account for as high as 40% of the total fruit weight (*3*), thus creating a significant byproduct source for value-added applications.

Interest in bioactive phytochemicals has increased in recent years due to their radical-scavenging and purported disease-prevention properties (*4−7*), and extracts from byproducts such as muscadine grape have potential to increase crop value and provide a functional food ingredient to the food industry (*8*). The polyphenolic composition of muscadine grape pomace is an attractive source for commercial exploitation. Composed of free ellagic acid, ellagic acid glycosides, ellagitannins, flavonols, and anthocyanins, predominantly as 3,5-diglucosides of delphinidin, cyanidin, and petunidin (*9*), the skins are an excellent antioxidant source from either wine or juice pomace. Muscadine grape pomace is a good source of valuable phytochemicals provided that extraction, handling, and stability issues are sufficient for value-added applications. Consequently, the purpose of these studies was to determine factors associated with the recovery, stability, and concentration of polyphenolics obtained from pomace of juice manufacture. Individual and total polyphenolics were monitored as indicators of extraction efficiency and stability in both laboratory- and pilot-scale trials that simulate potential commercial applications.

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MATERIALS AND METHODS

Preparation and Storage. Red muscadine grapes (cv. Noble) were used for both laboratory- and pilot-scale trials. For the laboratory-scale trial, whole muscadine grapes were obtained from a local vineyard in central Florida. Muscadine grapes were hand-harvested, and the pomace was retained following juice pressing and seed removal. To create variations in grape pomace composition, grape pomace was obtained following a hot-press juice production, obtained by crushing and heating of fresh grapes for 30 min at 70 °C prior to juice pressing. Pomace was also obtained from grapes that were crushed without removal of field heat (approximately 32 °C) and immediately pressed for juice (cold-press). For the pilot-scale trials, Noble grape pomace was obtained from a commercial juice processor (Paulk Vineyards, Wray, GA) using previously frozen grapes that were deseeded to produce a pomace that contained only skins. Juice production followed standard industry practices as described previously (*10*). The resulting pomaces from both laboratory- and pilot-scale trials were held at −20 °C for 30 days prior to extraction for polyphenolic recovery.

Extraction of Polyphenolics. To quantify total polyphenolics in the laboratory-scale trial, pomace was extracted twice with 100% methanol (1:1 ratio) to solubilize 100% of the polyphenolics and used for comparison of extraction efficiencies to solvent-free extracts. Aqueous pomace extracts (hot and cold press) were created in duplicate by soaking 1 kg portions of pomace in water (1:1 ratio) containing 3% citric acid (pH 2.1) for 24 h at 20 °C to extract polyphenolics by osmotic equilibration. Each pomace was manually pressed through cheesecloth to remove insoluble solids and clarified by vacuum filtration through Whatman no. 4 filter paper. For absorption to the solid phase supports, hot- and cold-press pomace extracts were diluted with aqueous 3% citric acid solution to an equivalent absorbance value at OD 520 nm. Dilutions were made to create suitable conditions for adsorption to Amberlite XAD-4 resins (Amberlite) and reversed phase C_{18} cartridges (C_{18}) and for subsequent stability trials. Amberlite (mesh size 20−60; Sigma Chemical Co., St. Louis, MO) resin (10 g) was previously conditioned with methanol, loaded into a glass column, and thoroughly rinsed with deionized water before adsorption. Similarly, 5 g Sep-Pak C_{18} mini-columns (Waters, Milford, MA) were conditioned with methanol and rinsed with deionized water before adsorption. Diluted extracts where divided into three equal portions with one retained without further modification (control), another adsorbed onto Amberlite over a 2 h period, and a third adsorbed onto C_{18}. Polyphenolics were eluted with 100% methanol, solvent was removed under reduced pressure at 40 °C, and the residue was redissolved in 3% citric acid solution. Storage stability was subsequently assessed in 15 mL glass tubes, flushed with nitrogen, and held at 20 and 30 °C for 0, 10, 30, and 55 days.

Pilot-scale trials were conducted to determine the effects of extraction and concentration on the recovery and stability of muscadine grape polyphenolics. Pomace (23 kg) from the commercial grape juice manufacture was extracted in 45 L of hot water (95 °C) for 3.5 h with mixing cycles (3 min) every 15 min to accelerate polyphenolic extraction. Pomace was pressed in a hydraulic press (500 bar), and insoluble solids were removed by filtration through cheesecloth followed by extract clarification through a 2 cm bed of diatomaceous earth under vacuum (Celite 545 Filter Aid, Fisher Scientific, Suwanee, GA). The clarified extract was divided into three fractions including one for fermentation and two for solid phase extraction as previously described. An aerobic fermentation was carried out following inoculation with wine yeast (*Saccharomyces cereviseae* strain Premium Cuveé) at a rate of 2.5 g/L at 25 °C until soluble solids decreased to a constant level monitored by refractive index and specific gravity. Following fermentation, the extract was again clarified through diatomaceous earth. The fermented extract was then evaporated by spray- or vacuum-drying and compared to the solid phase techniques. The spray-dryer (Anhydro, Copenhagen, Denmark) operated for 4 h at 220−230 °C with an exhaust temperature of 100−110 °C at a rate of 0.5 L of fermented extract per hour. For vacuum concentration, aliquots of the fermented extract were evaporated under reduced pressure at 40 °C for 40 min for complete moisture removal. After drying, the resulting powders were redissolved in 0.1 M citrate buffer at pH 3.0 to an equivalent anthocyanin-based color at OD 520 nm and held at −20 °C until analysis.

Chemical Analyses. Grape pomace was analyzed for polyphenolic content before and after aqueous extractions, before and after solid phase adsorption, and following the fermentation and drying procedures. Analyses included total ellagic acid and individual anthocyanins following acid hydrolysis as described elsewhere (*11*). Separations were made on a Dionex HPLC system and a PDA 100 detector using a Supelcosil LC-18 column (Supelco, Bellefonte, PA) and quantified using an ellagic acid (Sigma Chemical Co., St. Louis, MO) and cyanidin (Polyphenolics Laboratories AS, Sandnes, Norway) standards. Total ellagic acid accounted for contributions from free ellagic acid, ellagic acid glycosides, and ellagitannins as previously characterized in muscadine grapes (*12*) and was quantified simultaneously with six anthocyanidins. Storage stability of the extracts and isolates was determined by measuring total soluble phenolics using the Folin−Ciocalteu assay (*13*) with data expressed as gallic acid equivalents, total anthocyanins (*14*) with data expressed in cyanidin-3-glycoside equivalents, anthocyanin polymerization based on color retention in the presence of 5% sodium sulfite (*15*), and antioxidant capacity using the oxygen radical absorbance capacity (ORAC) assay (*16*) with data expressed in micromoles of Trolox equivalents per milliliter.

Statistical Analyses. In the laboratory-scale trial, data represent the mean of duplicate pomace isolations, each analyzed in triplicate, whereas in the pilot-scale trial, data represent the mean of three separate process replicates. Analysis of variance and means separations by LSD test ($P < 0.05$) were conducted using JMP5 software (*17*).

RESULTS AND DISCUSSION

Detailed phytochemical compositions of muscadine grapes and juice were evaluated in a series of previous investigations (*3*, *4*, *11*, *12*) with free ellagic acid, ellagitannins, anthocyanins, and flavonols identified as predominant compounds in this grape variety. Muscadine grapes contain several forms of ellagic acid including the unconjugated free form, ellagic acid glycosides, and ellagitannins. Anthocyanin content is primarily 3,5-diglucosides of delphinidin and cyanidin and was shown to be least stable to processing and storage (*18*, *19*). Red muscadine grape juice may be obtained by slowly heating grapes to activate native pectinase and facilitate juice and pigment removal (hot press), yet low or no heat may be applied for freshly harvested grapes to minimize bitter or astringent flavors (cold press). Muscadine grapes may also be frozen after harvest, and the freeze−thaw cycle is generally sufficient to facilitate juice extraction. Therefore, each processing method may create a pomace that significantly varies in phytochemical concentrations. Previous investigations revealed that from 19 to 78% of the total ellagic acid and from 12 to 32% of total anthocyanins were solubilized into hot-pressed juice depending on the cultivar (*9*). Remaining compounds in the pomace are commonly discarded after juice or wine production. Therefore, extractions and isolations of residual polyphenolics from muscadine grape pomace and their resultant stability during processing and storage were evaluated in an effort to assess their applicability in value-added applications.

Laboratory-Scale Trial. Grape juice (cv. Noble) pomace was evaluated prior to and following extraction with 3% aqueous citric acid to determine polyphenolic recovery rates. Extraction modifications were initially evaluated with each pomace to maximize phytochemical recovery and facilitate commercial utilization of the pomace. In these trials, extracts were made from 25% methanol in water, pectinase addition (Crystalzyme 100XL, 100 U/g, pH 3.8, 30 °C, Valley Research, South Bend, IN), and aqueous 3% citric acid. Following a 24 h static extraction no differences were observed for total anthocyanins among treatments (±5%, data not shown); therefore, extraction in acidified water was chosen because of its simplicity and lack of need for solvent removal and/or

enzyme treatment. Extraction with acidified water also left an intact grape skin with a residual polyphenolic concentration suitable for additional uses such as dry skin powders or fiber processing.

Evaluation of Polyphenolic Extraction from Muscadine Grape Pomace. The initial sugar content of the juice pomace ranged from 10 to 12% by refractive index and decreased by half following the 24 h extraction. Concentrations of total phenolics, anthocyanins, and total ellagic acid initially present in juice pomace compared to concentrations retained in the pomace after extraction with acidified water are reported in **Table 1**. Cold-pressed juice pomace contained higher initial phytochemical concentrations than hot-pressed juice pomace. Following extraction, water-based extracts from pomace contained from 30.5 to 41.4% of the total soluble phenolics, from 25.9 to 32.1% of the total ellagic acid, and from 36.3 to 62.0% of the total anthocyanins, whereas the rest was still held by the pomace. Longer extraction times (> 24 h) were deemed to be detrimental due to the potential development of spoilage organisms. The low extraction efficiency for total ellagic acid was attributed to the low solubility of free ellagic acid, whereas ellagic acid glycosides and ellagitannins are more water-soluble and were likely removed during the initial juice manufacturing procedures.

Factors that influence color values such as polymerization and naturally occurring copigments were not accounted for and resulted in extracts that contain from 106 to 137 mg/kg total anthocyanidins (**Table 2**). Adsorption to Amberlite resin served to isolate polyphenolics and remove sugars and other polar constituents for concentration purposes, and its storage stability was compared. The recoveries of total anthocyanidins and total ellagic acid before and after Amberlite adsorption are reported in **Table 2**. Amberlite resins are widely applied in food and environmental sciences to selectively remove or concentrate target species from aqueous trials (*20–26*). Anthocyanins were found to have excellent affinity to Amberlite because a 100% recovery from both extracts (cold and hot press) was observed, whereas total ellagic acid recoveries were 89 and 90% for cold press and hot press, respectively. This loss may be attributed to the lower affinity of ellagitannins to Amberlite-XAD4. Amberlite resins were previously used to isolate and purify ellagitannins from pomegranate husk (*26*), but different affinities for specific compounds are attributable to different particle sizes and/or surface affinities of these solid-phase materials.

Storage Stability of Polyphenolic Extracts from Muscadine Grape Pomace. The stability of the polyphenolic extracts was evaluated periodically over 55 days of storage at 20 and 30 °C and monitored for total soluble phenolics and total anthocyanins. Color degradation followed a first-order kinetic model where degradation rate constants (β_1) and half-life ($t_{1/2}$) for anthocyanin loss were calculated (*27–29*) as $\ln A_t/A_0 = -\beta_1 \times$ time, and $t_{1/2} = \ln 0.5/\beta_1$, where A_0 is the initial color absorbance value at 520 nm and A_t is the absorbance value at a given time (**Table 3**). The temperature quotients (Q_{10}) between extracts stored at 20 and 30 °C (*29*) for color stability ranged from 1.4 to 1.6. Anthocyanins were more stable when first partitioned from Amberlite compared to nonadsorbed extracts and resulted in an extended shelf life in both 20 and 30 °C. It was hypothesized that removing certain soluble compounds from the pomace extracts such as metal ions, soluble proteins, and sugars were influential in preventing anthocyanin degradation during storage. The effectiveness of Amberlite was confirmed as color degradation was delayed potentially due to the removal of soluble compounds from the stock matrix yielding improvements of 18.6–26.1% at 20 °C and 27.5–38.0% at 30 °C in color stability during storage, which was notable in pomace from both cold press and hot press (**Table 3**). Other studies have illustrated the deleterious effect of sugars on anthocyanins during both processing and storage (*27–29*).

Pilot-Scale Trial. Preliminary studies evaluated polyphenolic extraction from the muscadine grape pomace with different extraction durations and pomace to water ratios and utilized both hot (95 °C) and cold (20 °C) water to determine optimal extraction of target polyphenolics. Manually agitated hot water initially added at a 2:1 water to pomace ratio and allowed to stand for 4 h facilitated a more efficient extraction than cold water alone for longer exposure times, reducing the time for maximum polyphenolic extraction and potentially reducing levels of spoilage organisms. Longer extraction times following initial hot water exposure did not increase polyphenolic solubilization substantially as the water temperature rapidly cooled (temperature decreased from 95 to 25 °C in < 2 h). Furthermore,

Table 1. Average Concentrations of Polyphenolics in Muscadine Grape (Cv. Noble) Using Pomace Pre- and Postextraction Methods

		concentration (mg/L) after	
	extraction	cold-press treatment[a]	hot-press treatment[b]
total soluble phenolics	pre	2320 ± 175	1880 ± 230
	post	1360 ± 67.1	1310 ± 108
	% loss[c]	58.6	69.5
total ellagic acid	pre	776 ± 56.2	517 ± 138
	post	527 ± 113	383 ± 63.1
	% loss	67.9	74.1
total anthocyanins	pre	4050 ± 464	2200 ± 943
	post	1540 ± 196	1400 ± 230
	% loss	38.0	63.7

[a] Cold-press juice production (crushing of grapes right after harvest). [b] Hot-press juice production (crushing and heating of grapes for 30 min at 70 °C before pressing). [c] Polyphenolics retained in the pomace postextraction.

Table 2. Concentrations of Six Anthocyanidins and Total Ellagic Acid in Normalized Extracts during Amberlite XAD-4 Extraction from Muscadine Grape (Cv. Noble) Pomace As Affected by Processing Treatments

extract[a]	treatment	Dp (mg/L)	Cy (mg/L)	Pt (mg/L)	Pg (mg/L)	Pn (mg/L)	Mv (mg/L)	total anthocyanidins[b] (mg/L)	total ellagic acid (mg/L)
initial[c]	cold press[d]	39.2 ± 1.68	22.6 ± 0.65	31.8 ± 1.27	0.96 ± 0.06	17.1 ± 0.50	25.8 ± 0.39	137 ± 4.43 a[f]	96.9 ± 4.17 a
	hot press[e]	36.4 ± 2.11	14.5 ± 0.76	25.0 ± 1.69	0.42 ± 0.01	8.74 ± 0.96	20.5 ± 0.35	106 ± 5.18 a	73.8 ± 10.6 ab
after Amberlite adsorption	cold press	40.6 ± 5.76	20.4 ± 2.57	32.8 ± 3.84	0.85 ± 0.04	16.3 ± 0.66	28.1 ± 4.41	139 ± 17.3 a	86.4 ± 14.4 ab
	hot press	40.1 ± 4.91	17.1 ± 4.31	29.8 ± 6.33	0.69 ± 0.09	10.8 ± 2.70	27.4 ± 5.04	125 ± 23.3 a	66.4 ± 9.60 b

[a] Extracts were hydrolyzed into aglycones at 90 °C for 60 min in 2 N HCl containing 50% methanol prior to analysis. [b] Sum of individual anthocyanidins: (Dp) delphinidin, (Cy) cyanidin, (Pt) petunidin, (Pg) pelargonidin, (Pn) peonidin, and (Mv) malvidin. [c] Initial extracts were diluted not to exceed the absorbance capacity of Amberlite XAD-4. [d] Cold-press juice production (crushing of grapes right after harvest). [e] Hot-press juice production (crushing and heating of grapes for 30 min at 70 °C before pressing). [f] Values with similar letters within columns are not significantly different (LSD test; P < 0.05).

shortening extraction times reduced the likelihood of uncontrolled fermentations and other microbial growth.

Fermentation was evaluated as an alternative procedure to solid phase extraction techniques to eliminate bulk sugars from pomace extracts on a commercial scale in a solvent-free environment. Fermentation was then followed by spray- or vacuum-drying to remove water. By applying a fermentation step, a majority of sugars could be eliminated within 24–36 h with minimum loss of target polyphenolics. Therefore, preliminary studies evaluated the efficiency of fermentation before and after skin pressing. Fermentation rates were found to be faster when pomace was first pressed to obtain an extract. This difference was likely due to increased yeast motility through pomace extract. Aerobic fermentation (∼36 h) was sufficient to significantly reduce the sugar content without decreasing the polyphenolic content. Secondary (anaerobic) fermentation was not allowed to occur in these extracts, avoiding the development of unwanted volatile compounds that may have affected organoleptic properties. Thus, fermentation after skin pressing was utilized as a procedure to reduce the sugar content. Spray- and vacuum-drying were used as methods to eliminate water in fermented extract. The effects on polyphenolic concentration of these two treatments following fermentation were compared to solid phase concentration techniques.

Polyphenolic Stability As Affected by Concentration Processes. Total soluble phenolics, total anthocyanins, polymeric anthocyanins, and antioxidant capacity were assessed as markers to determine processing effects during extract concentration (**Table 4**). During fermentation the sugar content was reduced to 0.8%, whereas total polyphenolics, total anthocyanins, and anthocyanin polymerization remained unaffected. On the contrary, a reduction of 16.3% was observed in the antioxidant capacity of the extract. Although antioxidant capacity was reduced during fermentation, values were comparable to those of various fruits such as cherries (33.4 ± 3.4 μmol of TE/g) and

strawberries (35.4 ± 4.2 μmol of TE/g) and vegetables such as red cabbage (31.5 ± 6 μmol of TE/g) (*30*), showing important metal-reducing and radical-scavenging properties after processing. Results illustrated the ability of polyphenolics to withstand aerobic fermentation conditions without significant changes due to oxidation.

Two drying methods (spray- and vacuum-drying) were evaluated as means to concentrate polyphenolics from the fermented extracts, and their stability was assessed and compared to the initial fermented extract (**Table 4**). Anthocyanin concentration, total soluble polyphenolics, and antioxidant capacity were preserved during vacuum-drying because temperatures over 40 °C were avoided by the use of low pressure. Conversely, spray-drying had significant effects on total anthocyanins, total polyphenolics, and antioxidant capacity, indicating losses of 30.3, 21.5, and 23.3%, respectively. Polymerization of anthocyanins present in the extract was influenced by processing because the initial extract had the lowest concentration of polymerized anthocyanins (7.65 ± 0.52%), whereas spray-dried and vacuum-dried samples almost doubled the concentration of these compounds (14.3 and 14.8%, respectively). A correlation (r = −0.73) between the concentration of anthocyanins and polymeric anthocyanin content was observed in agreement with Weinert et al. (*31, 32*) who correlated processing with degradation or polymerization of anthocyanins.

In parallel, vacuum-drying following solid phase concentration techniques resulted in lower concentrations of soluble polyphenolics and anthocyanins, as well as a significantly lower antioxidant capacity than the original extract (**Table 4**). Reductions in total soluble phenolics and total anthocyanins were even larger than those determined following drying techniques applied to fermented extracts. The largest loss of antioxidant capacity (51.3%) was recorded in C_{18}, whereas Amberlite resulted in a 34.1% loss. Processing illustrated an increase in the polymerization of anthocyanins because all samples following drying procedures showed a significant increase in the polymer anthocyanin fraction compared to the initial extract (7.65%). The largest formation of polymers was induced by C_{18} followed by Amberlite and both drying procedures following fermentation. The extensive loss of color in both C_{18} and Amberlite procedures (32 and 41%, respectively) presumably occurred while anthocyanins were exposed to high-pH environment because negligible anthocyanin concentration was found in the unbound fraction from C_{18} and < 1.5% was found in the unbound fraction from the Amberlite resin. The stability of anthocyanins is known to be jeopardized by lowering the concentration of anthocyanins in a medium (*33*) and decreasing the acidity in their environment (*34*), possibly explaining why both affinity column isolation protocols decreased anthocyanin concentration in muscadine pomace extract. Samples subjected to Amberlite resin also showed the highest total soluble polyphenolic losses (**Table 4**). This can be explained by the

Table 3. Kinetic Parameters of Anthocyanin Degradation during Storage at 20 and 30 °C in Different Sources of Muscadine Grape (Cv. Noble) Pomace

		20 °C		30 °C		
isolate	treatment	$\beta_1{}^c$	$t_{1/2}{}^d$	β_1	$t_{1/2}{}^e$	$Q_{10}{}^f$
stock	cold press[a]	22.8	30.5 c[g]	36.4	19.1 *c	1.6
	hot press[b]	28.1	24.6 d	38.0	18.2 *d	1.4
Amberlite XAD-4	cold press	18.6	37.2 b	27.0	25.7 *b	1.4
	hot press	17.9	38.8 a	26.1	26.5 *a	1.5

[a] Cold-press juice production (crushing of grapes right after harvest). [b] Hot-press juice production (crushing and heating of grapes for 30 min at 70 °C before pressing). [c] Reaction rate constants ($\beta_1 \times 10^3$ days^{-1}). [d] Half-life (days) of initial anthocyanin content. [e] Asterisk (*) indicates a significant effect (LSD test; $P < 0.05$) due to storage temperature. [f] Temperature dependence quotients of color degradation as affected by increments in reaction temperature from 20 to 30 °C. [g] Values with similar letters within columns are not significantly different (LSD test; $P < 0.05$).

Table 4. Concentrations of Total Soluble Phenolics, Total Anthocyanins, Polymeric Anthocyanins, and Antioxidant Capacity in Muscadine Grape (Cv. Noble) Pomace Extract As Affected by Fermentation and Drying Protocols

process	total phenolics (mg/kg)	total anthocyanins (mg/kg)	polymeric anthocyanins (%)	antioxidant capacity (μmol/mL)	total ellagic acid (mg/L)
extraction	1640 ± 18.1 a[d]	1470 ± 102 a	7.65 ± 0.52 d	34.3 ± 0.57 a	168 ± 31.9 ab
fermentation	1580 ± 104 a	1520 ± 87.8 a	9.75 ± 0.77 cd	28.7 ± 2.53 b	176 ± 51.4 a
spray-drying[b]	1240 ± 48.4 b	1060 ± 26.3 b	14.3 ± 2.51 bc	22.0 ± 0.75 c	122 ± 6.77 bc
vacuum-drying[b]	1620 ± 56.7 a	1450 ± 55.9 a	14.8 ± 2.42 b	28.8 ± 1.23 b	202 ± 20.6 a
RP C_{18}[c]	1130 ± 43.8 c	996 ± 24.4 bc	25.8 ± 3.25 a	16.7 ± 0.56 d	123 ± 2.93 bc
Amberlite XAD-4	1026 ± 17.8 d	873 ± 78.9 c	18.6 ± 4.34 b	22.6 ± 0.90 c	113 ± 11.2 c

[a] Sum of individual anthocyanidins: delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin. [b] Spray- and vacuum-drying were applied after fermentation. [c] Reversed-phase C_{18} cartridge. [d] Values with similar letters within columns are not significantly different (LSD test; $P < 0.05$).

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presence of ellagic acid and ellagic acid derivatives (~10 mg/kg) in the unbound fraction of Amberlite (data not shown). These polyphenolics did not bind with the resin and represented 5.8% of the initial ellagic acid concentration.

This study illustrated that polyphenolics of muscadine grape pomace can be extracted by osmotic equilibrium in water, and this extraction can be accelerated by the use of hot water and agitation. The removal of organic acids and sugars from the extract delayed the color degradation during storage. Fermentation was an effective method to reduce the sugar content from the matrix with minimum changes to polyphenolic compounds, and vacuum-drying proved to be a suitable approach to concentrate polyphenolics, preserving their antioxidant capacity following fermentation. Although some antioxidant capacity was lost during processing, a significant amount was still detected in the final concentrate, thus showing promising results for future products from this proposed operation. Finally, the muscadine grape industry can expand their market and add value by processing polyphenolic extracts or dried skin materials using the byproducts from both juice and wine processing.

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