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## Chapter 11

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# Thermal Stability of Major Classes of Polyphenols in Skins, Seeds and Stems of Grape Pomace

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## Abstract

Grape pomace (GP) is a by-product of wine industry, in which remains significant amount of polyphenols. The freshly pressed pomace is perishable and has to be dried for use at later time. Depending on the drying methods, significant loss in polyphenols may occur during drying. This chapter describes the impacts of dehydration methods such as room temperature drying and vacuum drying on the retention of polyphenols in the pomace of Cabernet Sauvignon and Muscadine Nobel grapes using freeze dried sample as control. Seeds, skins and stems were separated manually after drying and the effects of drying methods on the stability of GP polyphenols were evaluated by the retentions of total polyphenol (TP), total anthocyanins (TA) and total flavonoids (TF). Results show that seeds and stems from both grape varieties were rich in TP and TF but low in TA, while skins were rich in anthocyanin. Compared to freeze drying, both room temperature and vacuum oven drying methods resulted in significant loss of TP, TA and TF in different parts of Muscadine pomace. Among different groups of polyphenols, anthocyanin was affected the most by drying methods. Flavonoids in Cabernet pomace exhibited higher heat stability than that in Muscadine pomace indicating the differences in flavonoid composition between the two grape varieties. Therefore, the polyphenol composition and stability of GP are variety dependent. The selection of drying method needs to consider what type of polyphenol to be preserved, variety of GP and the cost of drying method.

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**Keywords:** Grape Pomace; Thermal Stability; Drying Methods; Total Polyphenol; Total Anthocyanin, Total Flavonoids

## Introduction

Grapes offer a richer polyphenol profile than many other fruits. Grape pomace (GP), the residue of grape after wine making, remains significant amount of polyphenols. The health benefits of GP polyphenols have been the great interest of researchers, food manufacture and nutraceutical/pharmaceutical industry. Grape seed procyanidin extract (GSPE) modulates dyslipidemia associated with a high-fat diet in rats and repress genes controlling lipogenesis and VLDL assembling in liver (Baiges et al., 2010). Low dose (25mg per kg body weight per day) GSPE treatment of high-fat-diet (HFD) fed rats significantly reduced the adiposity index and the weight of all the white adipose tissue depots and reversed the increase in plasma phospholipids induced by the HFD feeding (Caimari et al., 2012). Chronic consumption of grape phenolics has been shown to reduce obesity development and related metabolic pathways including adipokine secretion and oxidative stress in a rat model (Décordé et al., 2009). Cancer chemopreventive and anticancer efficacy of grape seed extract and other grape-based products were summarized by Kaur et al., (2009). Grape seed extract was studied for use as an additive to inhibit the oxidation of meat products (Mielnik et al., 2006; Brannan and Mah, 2007) and bread (Peng et al., 2010).

GP is composed of grape skin, seeds and stem, and is rich in different type of extractable phenolic antioxidants (10-11% of dry weight) (Makris, et al., 2007). The grape skins, part of pomace, are proven to be rich sources of anthocyanins, hydroxycinnamic acids, flavanols, and flavonol glycosides, whereas flavanols were mainly present in the seed portion (Kammerer et al., 2004). The content of total phenolics of grape skin and grape seeds varies with grape variety, climate condition, soil type, and maturity (Fuleki 1997; Shi, 2003; McCalluma et al., 2007; Montealegre et al., 2006). Because grapes are seasonal agricultural products and the freshly pressed pomace is perishable and has to be dried or stored at freezing temperature for use at later time. Depending on the type of drying methods, significant loss in polyphenols may occur during drying.

The stability of fruit polyphenols under some thermal processing condition was studied by many researchers. Larrauri et al., (1997) reported that drying of grape seeds at 100 and 140 °C resulted in 18.6 and 32.6% reduction of extractable total polyphenols, respectively, and reduced antioxidant activity of grape seeds compared with freeze drying. It was also reported that some thermal processing methods increased the extractability of polyphenols from plant matrix. Roasting of peanuts and almonds before decoating not only increased total extractable phenolics in the skins, but also resulted in changes of phenolic composition of the skin extracts, for example, it increased procyanidin oligomers and decreased monomers (Yu et al., 2007; Garrido et al., 2008). Extrusion processing has been found to increase small molecule procyanidins in sorghum, grape seed, and grape as well as blueberry pomace while decreasing the procyanidin oligomers or polymers in the process (Khanal, 2009a; Khanal, 2009b). Dry heating of freeze dried GP in a forced air oven at 40, 60, 105, and 125 °C for 72, 48, 16, and 8 hrs respectively decreased anthocyanin and procyanidin concentrations significantly, except when heated at 40 °C for 72 hrs. The reduction of procyanidin occurred when heated at 60 °C

or above with no further reduction when heating temperature increased from 105 to 125 °C, and total anthocyanin loss was highest at 125 °C (Khanal et al., 2009b and Khanal et al., 2010). Phenolic extracts of thermal processed whole and powdered grape seeds had higher *in vitro* antioxidant activity than those of untreated grape seeds (Kim et al., 2006).

However, the studies mentioned above were conducted at very high temperature. No significant loss of both procyanidin and anthocyanin was observed when heated at 40 °C for up to 3 days (Khanal et al., 2010). Polyphenols are very sensitive to UV light (Shi et al., 2003), sundry is usually not used to dry polyphenol rich products. Freeze drying preserve the bioactive compounds but the cost is very high. Drying at mild temperature such as vacuum drying is faster and more cost effective than freeze drying, but the changes in polyphenol composition due to vacuum drying of GP has not been reported. The time needed to dry GP and the retention of polyphenol at room temperature (about 25°C) have not been reported either. This chapter focused on the stability of GP polyphenols under mild drying condition such as room temperature drying under dim light and vacuum drying under dim light using freeze drying as control. The composition and stability of polyphenols in Cabernet Sauvignon and Muscadine Noble were compared.

## Materials and Methods

### Materials

Grape pomaces (GP) The pomaces of two grape varieties/cultivars were obtained from two North Carolina wineries. Fermented Cabernet Sauvignon pomace was provided by Grove Winery (Gibsonville, NC, USA), while fermented Muscadine Nobel pomace was provided by Benjamin Vineyard and Winery (Graham, NC, USA). Pomaces were picked right after pressing and were stored in refrigerator before drying.

Chemicals: Folin-Ciocalteu reagent, gallic acid and (+)-catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA), other chemicals including reagent ethanol, acetonitrile (HPLC grade), acetic acid, sodium nitrite, sodium acetate, aluminum chloride and hydrochloric acid were purchased from Fisher Scientific (Atlanta, GA, USA).

### Dehydration of Grape Pomace

The wet GP from each grape variety was divided into three portions. Each portion was spread as 1.0 inch thick in a tray. One portion was dried at 70°C in an Isotemp vacuum oven (Fisher Scientific, Ashville, NC USA) for 24 hrs. Another portion was frozen at -22°C for 48 hrs, and then dried using a FreeZone 6 Liter Freeze Dryer (Labconco, Kansas City, MO USA) for 48 hrs; the rest was dried in a well ventilated room for one week at 22°C. The seeds, skins and stems of dry GP were manually separated and weighed. The ratios of seeds, skins and stems to total dry pomace were calculated. They are then ground into fine powders using a coffee grinder. The moisture contents of ground samples obtained from different drying methods were determined by drying the ground pomace at 80°C for 24 hrs using the vacuum

oven. Powders passed through 40 mesh sieves were packed separately in bottles and stored at 4°C until use.

### Polyphenol Extraction

Crude polyphenol was extracted using 70% ethanol/water solution (v/v). Briefly, 10.00g of dry seeds/skins/stems powder was weighed into a 250 ml amber flask, and 100 ml of extraction solvent was added to the flask. The mixture was stirred for 1 hr at room temperature under dim light, and then centrifuged at 3000g for 15 min. The resulting supernatant was collected and the residual solid was extracted one more time using the same procedure. Supernatants were combined and centrifuged again to remove any particles. The volume of extract was recorded. The final supernatants were stored at -22°C for polyphenol analysis. All extractions and determinations were conducted in triplicate and results were expressed as mean±standard deviation.

### Determination of Total Phenolic Content (TP)

The total polyphenol (TP) contents of the extracts were determined by a modified Folin-Ciocalteu method (Singleton et al, 1999). Briefly, 20 µl of properly diluted the sample solution, 1.28 ml of distilled water and 100µl Folin-Ciocalteu's reagents (Sigma-Adrich, St. Louis, MO USA) were mixed and let stand for 8 minutes at room temperature. A volume of 600µl of 10%NaCO<sub>3</sub> was added. Deionized water was used as blank to replace 20µl of sample.

The mixture was allowed to stand for 2 hrs at room temperature under dim light or at dark to develop color. The absorbance was measured at 765 nm using a Genesys™ 10 Spectrophotometer (Spectronic Unicam, NY). The TP concentration was calculated according to the calibration equation developed using gallic acid as standard, and expressed as gallic acid equivalents (mg GAE/g dry pomace) according to the dilution factor, total volume of extract and weight of pomace. The linear range of the calibration curve was 50 to 1000 µg/ml (r = 0.99).

### Determination of Total Anthocyanins (TA)

The TA concentration of extract was determined by AOAC method 2005.02 (AOAC, 2006). Extracts were quantitatively diluted (1:4, v/v) with pH1.0 buffer (0.25M potassium chloride) and pH 4.5 buffer (0.4M sodium acetate), respectively. The absorbance of test portion diluted with pH 1.0 buffer and pH 4.5 buffer were measured at both 520 and 700 nm within 20-50 minutes of preparation using deionized water as blank. Anthocyanin concentration was calculated using the equation below and expressed as cyanidin-3-glucoside equivalents.

$$\text{Anthocyanin (cyanidin-3-glucoside equivalents, mg/L)} = (A \times MW \times DF \times 10^3) / (\epsilon \times L)$$

where  $A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH 1.0}} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH 4.5}}$ ; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; L = path length in cm;  $\epsilon = 26,900$  molar extinction coefficient, in  $\text{L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ , for cyd-3-glu; and  $10^3 =$  factor for conversion from g to mg. The total anthocyanin content in dry pomace was expressed as mg/g dry pomace, according to extract volume and sample weight.

### Determination of Total Flavonoid Content (TF)

The TF content was determined using a colorimetric method described previously by Xu and Chang (2007). Briefly, a dose of 0.25 ml of the GP extract or (+)-catechin standard solution was mixed with 1.25 ml of distilled water in a test tube, followed by adding 0.75  $\mu\text{l}$  of 5%  $\text{NaNO}_2$  solution. After 6 min, 150  $\mu\text{L}$  of a 10%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  solution was added and allowed to stand for another 5 min before adding 0.5 mL of 1M NaOH. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank (the same mixture without the sample) at 510 nm using a Genesys™ 10 Spectrophotometer (Spectronic Unicam, NY). The results were expressed as microgram of (+)-catechin equivalents ( $\mu\text{g}$  CAE/g sample) using the calibration curve developed from (+)-catechin. The linear range of the calibration curve was 10-1000  $\mu\text{g}/\text{ml}$  ( $R^2=0.9978$ ).

### HPLC Analysis of GP Extracts

A Waters HPLC system consisted of In-Line Degasser AF, 717 Autosampler, 1525 Binary HPLC Pump, and 2748 Dual Wavelength Absorbance Detector was used to monitor the changes of individual polyphenol caused by different drying methods. All supernatants were diluted using same dilution factor then filtered by PVDF syringe filters (0.2  $\mu\text{m}$ ) before injection. The Nucleosil RP  $\text{C}_{18}$  column (250 mm x 4.6 mm, particle size 5  $\mu\text{m}$ ) was used as stationary phase. The samples were eluted by gradient elution mode using binary mobile phase A (2% acetic acid in DI water) and B (100% acetonitrile) at flow rate of 1.0 ml/min. In this mode B was increased from 5% to 75% in 20 min, to 100% in another 5 min, maintained at 100% for 5 min, returned to 75% in 5 min, and then to the initial condition (5%) in 5 min and remained for 5 min before next injection. Peaks eluted were detected at wavelength of 280 nm. Peaks of different samples at same retention time were overlaid for peak height comparison.

## Results and Discussion

### Moisture Retention of GP Dried by Different Methods

Table 1 shows that the moisture contents of GP dried by different methods differ slightly. The freeze dried samples had lowest moisture while the room temperature dried sample had highest moisture for both Muscadine and Cabernet pomace. Data shows that vacuum oven

drying for one day removed more moisture from wet GP than room temperature drying for 7 days. Therefore, vacuum oven drying is much more efficient than freeze drying and room temperature drying.

**Table 1. Moisture contents of grape pomace dried by different methods**

Drying method	Muscadine	Cabernet
	Pomace	Pomace
Freeze drying (48hrs)	3.37±0.23	3.54±0.30
Room Temperature drying (1 week)	7.03±0.16	5.05±0.21
Vacuum oven drying (24 hrs)	4.79±0.14	4.36±0.12

### Contribution of Seeds, Skins and Stems to the Total Extractable Polyphenols of GP

Table 2 summarizes the percentages of seeds, skins and stems in the dry GP and their contributions to the total polyphenol content of the pomace. Data were obtained from freeze dried samples.

**Table 2. The contribution of seeds, skins and stems in dry GP to the total extractable polyphenols of GP (freeze dried samples)**

	Muscadine Noble			Cabernet Sauvignon		
	% Dry weight	TP (mg/g)	% Contribution to TP of pomace	% DM	TP	% Contribution to TP of pomace
Seed	36.65	36.42	49.46	43.78	34.67	72.59
Skin	60.72	19.37	44.29	52.79	9.84	24.84
Stem	3.83	44.13	6.26	3.92	13.73	2.57
Total	101.10	25.98	100.01	100.00	20.91	100.01

The seeds, skins and stems contents were 36.65, 60.71 and 3.83% of the total dry weight of Muscadine Noble pomace, and 43.78, 52.79 and 3.92% of the total dry weight of Cabernet Sauvignon pomace, respectively. Although the skin contents were significantly higher than seed content, the latter contributed significantly higher percentage of polyphenols to the pomace, particularly in the Cabernet Sauvignon pomace because of the higher TP content of grape seeds. The contribution of stem to the TP of whole pomace was very limited due its small percentage in the pomace, although its TP content was the highest compared with other parts. Therefore, seeds and skins were the major contributors of GP polyphenol.

### Effects of drying methods on TP, TA and TF of grape pomace

Figure 1 shows that the effects of drying methods on the stability of GP polyphenols varied significantly with grape variety. Compared to freeze drying, both room temperature drying and vacuum oven drying resulted in significant loss of TP in each part of Muscadine

pomace but not Cabernet pomace. The TP in Cabernet seeds, skins and stems exhibited higher heat stability than that in Muscadine pomace.

The reasons of the observed result might include that 1) the polyphenols remained in Cabernet GP after alcohol fermentation were more stable than those in Muscadine GP, and 2) drying at higher temperature might increased the extractability of polyphenols (Kim et al., 2006) which resulted in relatively constant extractable TP content even some degraded during drying. Although the GP was dried at relatively low temperature in this chapter, the losses of TP in Cabernet and Muscadine seeds were comparable to that reported by Larrauri et al., (1997) that drying of grape seeds at 100 and 140 °C resulted in 18.6 and 32.6% reduction of extractable total polyphenols.

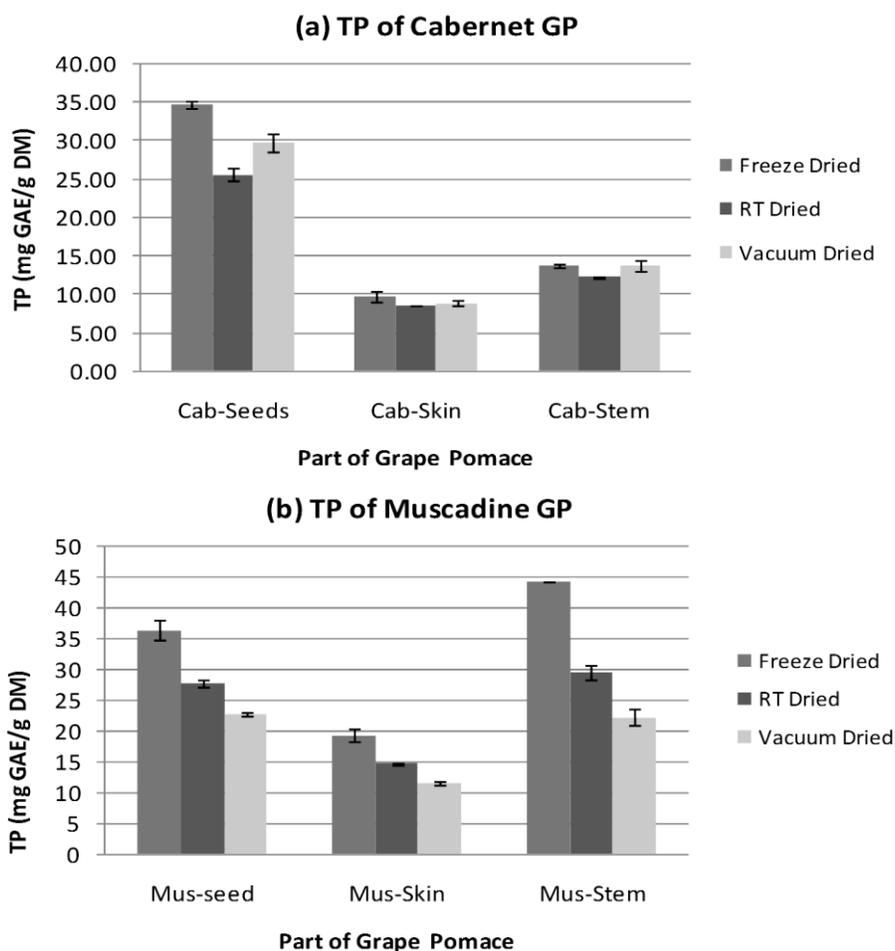


Figure 1. Effects of drying methods on total extractable polyphenol contents (TP) of GP seeds, skins and stems. (a) Cabernet Sauvignon pomace, (b) Muscadine Nobel pomace.

Comparing Figure 2a and Figure 2b, it can be seen that freeze dried Cabernet seeds and Muscadine seeds had similar total anthocyaning (TA) contents, but Muscadine skins and stems had much higher TA than Cabernet skins and stems. Drying at room temperature for 7 days and at 70°C vacuum oven for 24 hours caused significantly loss of TA in all parts of

both pomaces, and room temperature drying seems destroy more TA than vacuum drying due to longer drying time. The results are in good agreement with that reported by Khanal et al., (2009b; 2010). Therefore, the stability of GP anthocyanin is very low. The TA degrades significantly within the GP matrix even at room temperature, and faster drying under reduced pressure could preserve more TA than drying at room temperature for longer time.

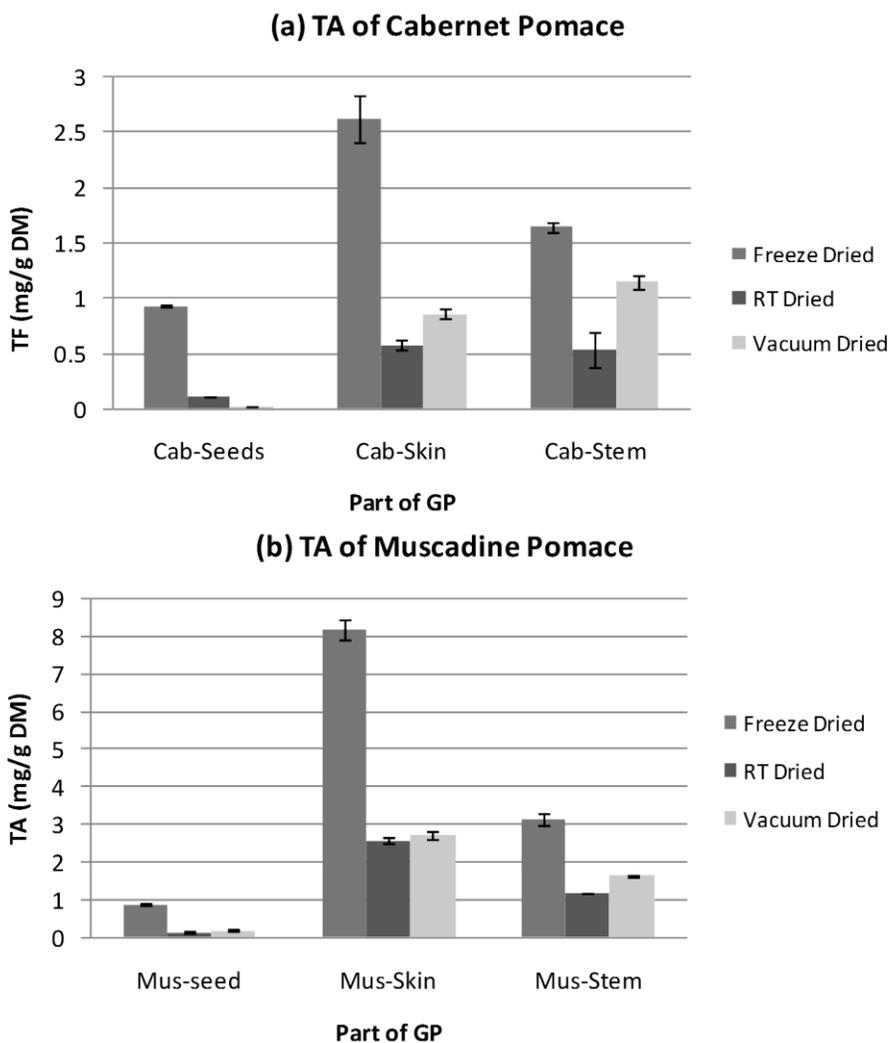


Figure 2. Effects of drying methods on total anthocyanin contents (TA) of GP seeds, skins and stems. (a) Cabernet Sauvignon pomace, (b) Muscadine Nobel pomace.

Figure 3 shows the changes of total flavonoids (TF) in GP due to different drying methods. In freeze dried samples, the TF contents of Cabernet seeds and skins were slightly higher than that of Muscadine seeds and skins, but the TF content of Cabernet stems is much lower than that of Muscadine stems. The room temperature and vacuum drying methods did not seem to cause TF loss in Cabernet seeds, but reduced TF in skins and stems significantly. This is because anthocyanins are a major polyphenols in the red/purple grape skins and stems while catechins and procyanidins are the major polyphenols in the grape seeds (Xia et al.,

2010). The TF contents in all parts of Muscadine pomace were dramatically affected by drying method. The results indicate that TF composition of Cabernet seeds is different from that Muscadine seeds. According to Mohd Zainol et al., (2009), catechin and rutin were the most stable flavonoids found in *Centella asiatica*. Therefore, catechins might be the major extractable flavonoids in Cabernet seeds, which contributed to the relatively higher thermal stability of TF in Cabernet seeds.

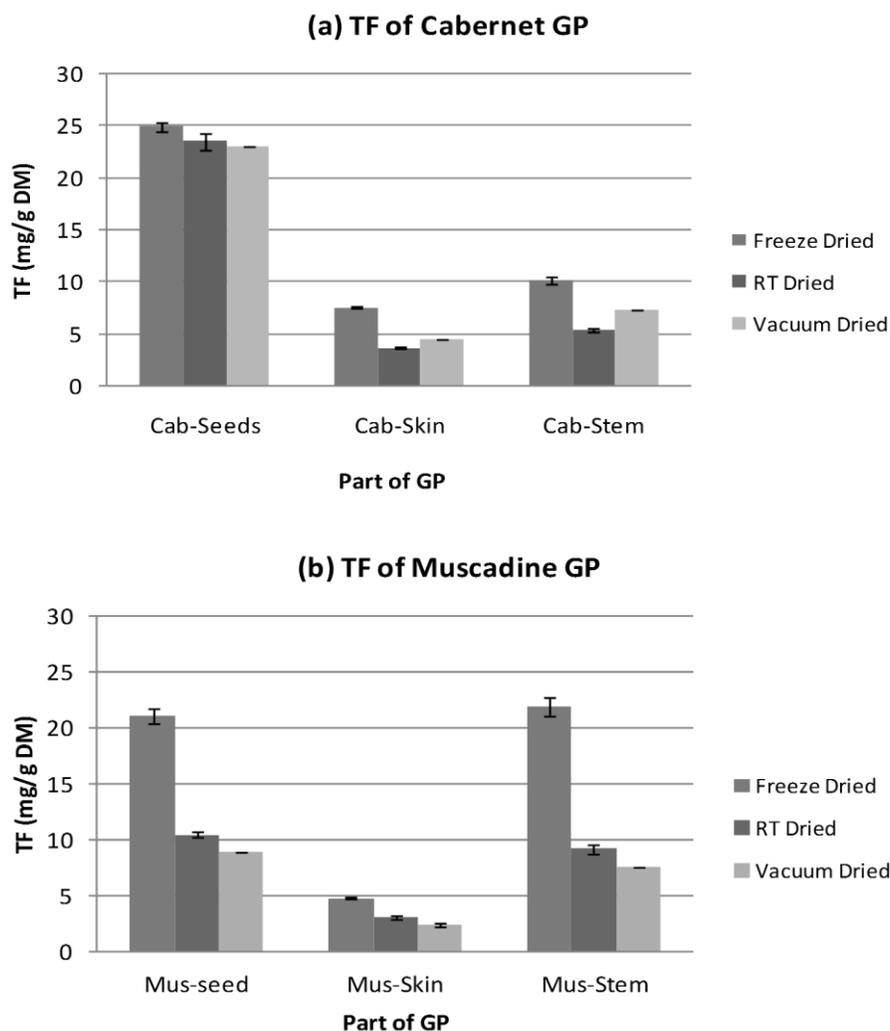


Figure 3. Effects of drying methods on total flavonoid contents (TF) of GP seeds, skins and stems. (a) Cabernet Sauvignon pomace, (b) Muscadine Nobel pomace.

### Changes of Individual Polyphenols Due to Drying

Above results indicate the differences in polyphenol composition and stability between the two grape varieties. HPLC analysis was conducted to view the changes of individual polyphenols in the pomace during drying. Due to the lack of mass detector and standards to

identify all individual polyphenols, major peaks separated by HPLC were numbered and their heights were qualitatively compared. Figure 4 summarized the effects of vacuum oven drying on the loss of individual polyphenols in skin, stem and seeds of the Muscadine Noble GP.

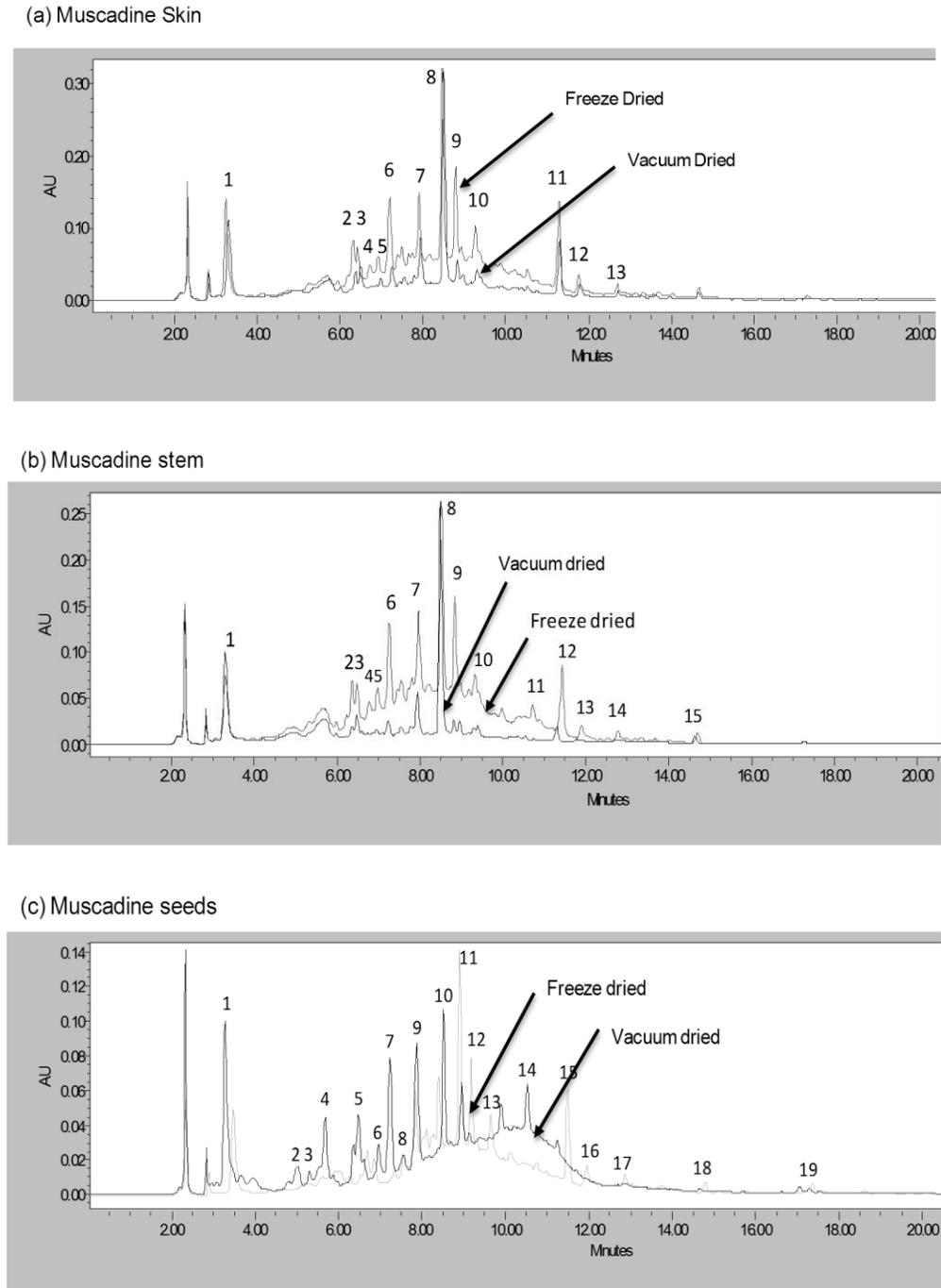


Figure 4 Effects of drying methods on individual phenolics in skin (a) stems (b) and seeds of Muscadine Nobel pomace.

Compared with freeze dried pomace, vacuum oven drying significantly reduced the heights of all peaks in the extracts of skins and stems with exception of peaks 1 and 8 (Figure 4a and Figure 4b). The peaks with significantly reduced heights might represent anthocyanins and anthocyanidins since they are very sensitive to heat as described above. The effects of vacuum drying on the polyphenols of grape seeds showed different pattern (Figure 4c). More peaks were resolved from seed extracts. The vacuum oven drying caused loss of peaks 11, 12, 13 and 15, but increased heights of peaks 1, 2, 3, 4, 5, 7, 9, 10 and 14 in the seed extracts of Muscadine pomace. This suggests that 1) drying at higher temperature (70°C) results in increased extractability of some polyphenols in the plant matrix as reported by Kim et al., (2006), but also destroyed some heat sensitive polyphenols; 2) degradation of large phenolic compounds such as oligomeric- or polymeric-procyanidins formed small molecule phenolics such as catechins as reported by Khanal et al., (2009a and 2009b). The net result is the lower TP, TA and TF in vacuum dried GP than that in the freeze dried GP.

## Conclusion

This chapter demonstrates that among different groups of polyphenols, anthocyanin was affected the most by drying methods. The effect of the drying method on TF depends on the variety of grape and the part of GP. While vacuum oven drying caused significant loss of TF in Muscadine pomace, it preserved more TF in Cabernet pomace, and the TF content in Cabernet seeds was only slightly affected by drying methods. Therefore, to select an appropriate drying method, it is important to consider the target polyphenols to be preserved and the variety of grape pomace. To preserve anthocyanin, freeze drying is required; to increase the extractability of some flavonoids, vacuum drying may be more suitable in terms of drying time and cost; room temperature drying does not need special equipment, but it takes significantly longer time than other methods, and it may also cause growth of mold if the drying environment is humid.

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