SHORT REPORT

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Changes of the Anthocyanins and Antioxidant properties of Concord Grape (*Vitis labrusca*) Pomace After Acid Hydrolysis

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Abstract: Grape pomace contains high levels of valuable antioxidants such as anthocyanins and phenolic compounds that help prevent chronic diseases such as cardiovascular problems and cancers. In this study, Concord grape pomace was soaked in acidic solutions at different time intervals and pHs in a water bath at 80 ^{o}C. Five kinds of anthocyanins were released and identified in the pomace after acid hydrolysis. The releasing rate of anthocyanins and antioxidant activities of the acid hydrolyzed pomace extracts were determined by multitest systems. Different antioxidant assays including total antioxidant capacity (TAC), total phenolic content (TPC) and free radical scavenging activity (RSA) were used to evaluate the antioxidant properties of the acid hydrolyzed pomace extracts. The change in antioxidant capacity of the pomace extracts during hydrolysis was correlated with total phenolic content and free radical scavenging activity but had little relationship with anthocyanin contents.

Keywords: Concord grape; *Vitis labrusca*; anthocyanin; antioxidant; phenolic; pomace.

1. Plant Source

Concord grape juice is a rich source of flavonoids including catechins, epicatechins, quercetins, anthocyanins, and proanthocyanidins [1,2], responsible for health beneficial antioxidant and anti-inflammatory effects [3]. The utilization of grape pomace as an economic source of bioactive components for meeting the public demand of safer food production has been investigated. Grape pomace, an industrial waste from the wine process, is composed mainly of grape seeds and skins. Grape seed has an estimated composition of 40% fiber, 16% essential oil, 11% protein, and 7% complex phenolic compounds by weight [4].

Significant amounts of bound anthocyanins and phenolics are retained in grape pomace [5]. The pomace can be accessible as an interesting new food ingredient by simple grinding process to reduce the coarse texture. The free anthocyanins rich pomace could then be used as a functional food ingredient or nutritional supplement with enhanced delivery of the anthocyanins and fiber from the pomace [6]. Pharmacological usable substances such as antioxidant components were obtained by extraction from industrial by-product grape pomace.

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Fresh Concord grape samples were obtained from a local grocery store (Wal-Mart Stores, Inc. (Bentonville, AR., USA)).

2. Previous Studies

In most previous pH liability studies of anthocyanins, the pH-differential spectrophotometric method was widely used in quantification. The absorbance of anthocyanins is very sensitive to pH which could easily cause deviations in quantification [6-9]. Although the variability in pH directly affects the structure and subsequent extractability of anthocyanins arising from the rich free acid/conjugate base equilibrium of these compounds [10], little effort has been spent on investigating the pH effect on extraction yield. The difference of TAC values measured before and after acid hydrolysis may directly reflect the amount of anthocyanins. Pomace had a high concentration of "cell wall-bound" phenolics [11]. Acid hydrolysis is an important step for the extraction of anthocyanins and other phenolics from the pomace samples by breaking down the bonds between phenolics and pomace matrix, allowing maximum release of phenolics.

3. Present Study

One kilogram of fresh grapes were ground in blender (Waring, Stamford, CT, U.S.A.) and transferred to a bucket. The blended grape slurry was filtrated using cheese cloth to obtain the grape pomace. Then, the pomace was mixed with 2.5 kg of distilled water in the bucket to bleach the pomace. After the mixture was agitated for 10 min, the water was decanted. The bleaching step was repeated until the wash water became clean and colorless. Then, the bleached anthocyanins pomace was spread on an aluminum foil and dried under a hood at room temperature. The dried pomace was stored at -4°C until use.

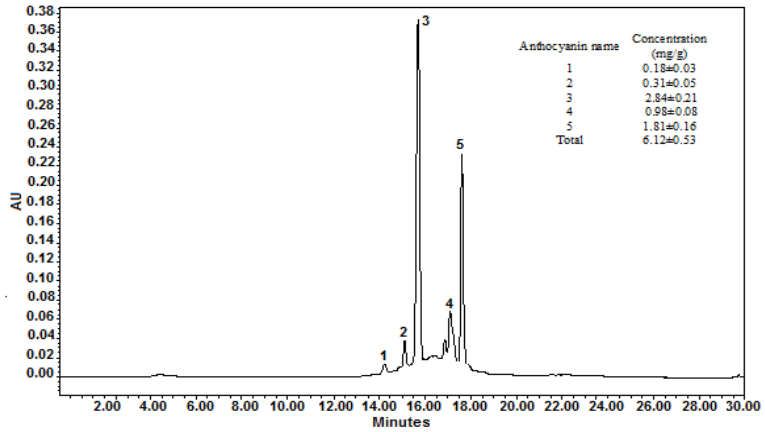

Figure 1. HPLC chromatogram of the anthocyanins released from grape pomace after hydrolysis at pH 1 for 30 min. Peaks: 1, delphinidin 3,5-diglucoside; 2, cyanidin 3,5-diglucoside; 3, delphinidin 3-glucoside; 4, cyanidin 3-glucoside; 5, petunidin 3-glucoside.

In this study, a sensitive HPLC method was used to determine the anthocyanin profile and levels. Five anthocyanins, namely delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, delphinidin 3-glucoside, cyanidin 3-glucoside and petunidin 3-glucoside were released from the completely bleached pomace after acid hydrolysis (Figure 1). Delphinidin 3-glucoside and petunidin 3-glucoside were the two major anthocyanins which constituted approximately 75% of the total anthocyanins. The anthocyanins released into solution increased from undetectable level to 0.67 mg/g at pH 1, 0.48 mg/g at pH 2, 0.45 mg/g at pH 3, 0.23 mg/g at pH 4, and 0.15 mg/g at pH 7, before heating (*i.e.*, at zero hydrolysis time) (Figure 2a). As shown in Figure 2a, lower pH hydrolysis was more effective for releasing bound anthocyanins than those at higher pHs. The anthocyanins content reached the highest level (6.12 mg/g of pomace) after 30 min of 80 ^{o}C acid hydrolysis at pH 1, while the corresponding

values were 5.22, 5.09, 4.45 and 3.95 mg/g for the samples at pH 2, 3, 4 and 7 (control), respectively, under the same conditions (Figure 2a). Similar results were reported by Patil *et al*. that the higher acidity of solution for extracting anthocyanins from red radish gave higher extraction yield [12]. Also, release rate of anthocyanin from pomace was found to increase with increasing acidity in boiling treatment, reported by Bener and coworkers [6].

The anthocyanins content began to decrease at 45 min (Figure 2a). It decreased to a similar level at the end of 60 min acid hydrolysis at 80 ^{o}C in pH 1, 2, 3, and 4 solutions. The results indicated that it was a dynamic process between the release and degradation of anthocyanins from grape pomace during acid hydrolysis. Temperature showed a double-fold effect of increasing the extraction rate by increasing permeability and diffusion coefficients, but at the same time increasing the possibility of degradation and polymerization of anthocyanins [13]; the result of these two conflicting factors in this work was the achievement of maximum extraction at 80 ^{o}C at the end of 30 min. The thermal stability of anthocyanins of red-flesh sweet potato in different pH solutions was studied by Cevallos-Casals and coworkers [14]. They found that the anthocyanins of samples at pH 3 were more resistant to heat processing than at pH 1. It was also reported that the anthocyanins in the blueberry pomace were degraded after 15 min of the boiling treatment and stability of anthocyanins decreased with increasing acidity [15]. These results were in accordance with our findings that hydrolysis in stronger acid solution could also accelerate the degradation of anthocyanins, although it was very effective in increasing the release of bound anthocyanins from the pomace, possibly as a result of carbinol-flavylium cation transition of anthocyanins upon acidification [16].

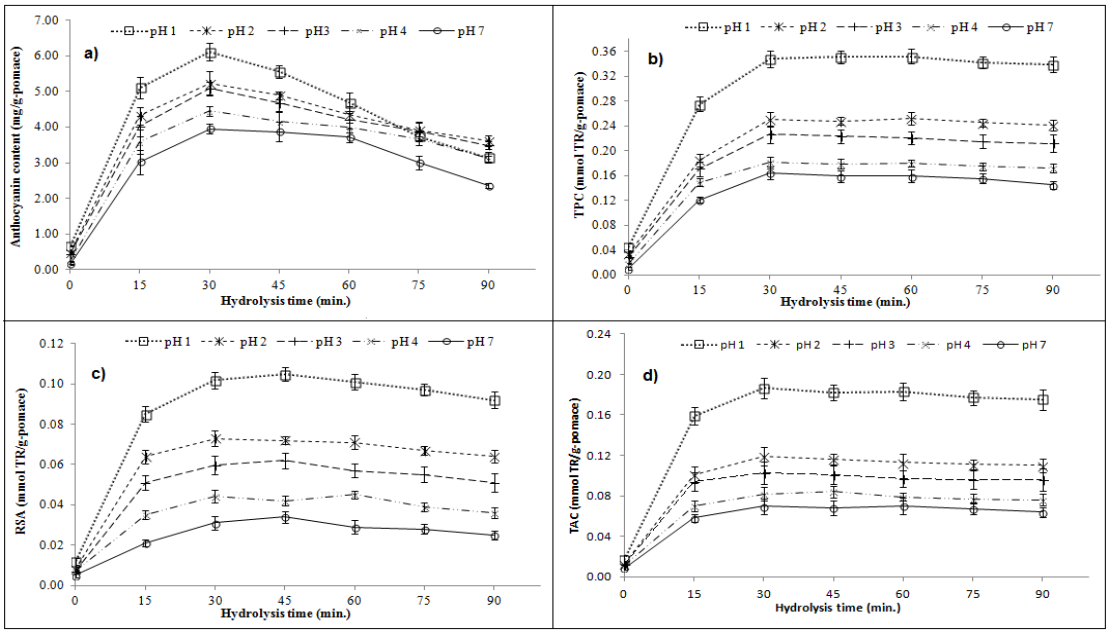

Figure 2. a) Anthocyanin content; **b)** Total phenolic content (TPC); **c)** DPPH free radical scavenging activity (RSA); **d)** CUPRAC total antioxidant capacity (TAC) of grape pomaces as a result of acid hydrolysis at 80 ^{o}C in different pH treatment and control solutions. Data with different letters are significantly different in their values at $P < 0.05$.

Changes of antioxidant properties of pomace during acid hydrolysis: Several studies reported that the antioxidant properties of food materials were well correlated with their total phenolic content [6,17,18]. Since Folin-Ciocalteu assay utilizes the phosphomolybdotungstate chromogen converted into molybdenum(V) blue upon e-transfer from antioxidants, it may also act as a TAC assay [19]. Changes of the total phenolic content (TPC) were investigated during acid hydrolysis at different acidic pHs. TPC reached the highest value after 30 min and 80 ^{o}C acid hydrolysis for all pH levels, and after 30 min, TPC was nearly maintained at the same level (Figure 2b). As opposed to the maximum observed for anthocyanins with respect to time, this result shows that total phenolics are essentially not degraded with time at elevated temperature and therefore are relatively more stable. As

shown in Figure 2b, TPC of hydrolyzed pomace in trolox (TR) equivalent units at pH 1, 2, 3, 4 and 7(control) after 30 min were 0.35, 0.25, 0.23, 0.18 and 0.16 mmol TR/g-pomace, respectively.

Antioxidant activity, especially free radical scavenging activity (RSA), has a great importance due to the deleterious role of free radicals in foods and biological systems [20]. Free radical scavenging activity of grape pomace samples was determined according to a modified version of the DPPH method introduced by Sánchez-Moreno *et al*. [21]. Similar to TPC, RSA reached the highest values after 30 min acid hydrolysis at 80 ^{o}C and essentially remained at a similar level, though minor reductions in RSA were observed beyond 45 min, possibly due to polymerization and condensation reactions decreasing the effective phenolic –OH groups. It has also recently been established that the DPPH radical does not respond well to phenolic polymers due to steric accessibility problems [22]. The DPPH$^{\bullet}$ scavenging activity of the hydrolyzate decreased with increased pH (Figure 2c). The highest RSA (0.10 mmol TR/g-pomace) was obtained with pH 1 solution for 30 min acid hydrolysis at 80 ^{o}C.

Total antioxidant capacity (TAC) was determined by the modified CUPRAC method described by Bener *et al*. [23], based on the reduction of copper(II)-neocuproine (Cu(II)-Nc) to the orange-colored Cu(I)-Nc chelate complex on a Nafion membrane sensor. As shown in Figure 2d, TAC reached the highest values after 30 min acid hydrolysis for all pH levels. TAC of hydrolyzed pomace increased with increasing acidity of solution where the highest value was noted for pH 1 hydrolyzed sample. TAC of pH 1, 2, 3, 4 and 7 hydrolyzed pomace were found as 0.19, 0.12, 0.10, 0.08, and 0.07 mmol TR/g-pomace, respectively. Like Folin-Ciocalteu, the CUPRAC method may be regarded not to suffer from steric accessibility of phenolic polymers because the absorbances remained relatively stable for extended periods of hydrolysis (Figures 2b,d).

Relationships of the Anthocyanin Content and Antioxidant Properties of Concord Grape Pomace After Acid Hydrolysis: There was a low correlation between TAC and anthocyanins released during acid hydrolysis. The linear regression coefficient regarding the change of phenolic content *versus* the change of anthocyanins content was 0.6358 (Figure 3a). This weak correlation is in accordance with the findings of Gomez-Plaza *et al*. who reported that FRAP antioxidant capacity and anthocyanin content of grape pomace did not correlate significantly [16]. The possible reason for this behavior is that anthocyanins merely constituted a fraction of total antioxidative polyphenols, and a part may not always correlate significantly with the whole. Similar to the anthocyanins content, TAC slightly decreased with extended time acid hydrolysis process. Compared with the decrease of anthocyanins content, the decrease of phenolics content was relatively slow. The reason may be that some anthocyanins could convert to other phenolic compounds after the 30 min acid hydrolysis. In the study of Bener *et al*. [6], the amounts of some antioxidant compounds such as protocatechuic acid and catechin contents were observed to increase as a degradation product of anthocyanins after the long-term boiling hydrolysis process for blueberry pomace. Rupasinghe also reported that protocatechuic acid is a major degradation product of anthocyanins [24].

There was a strong correlation between total antioxidant capacity, total phenolic content and free radical scavenging activity in the pomace as a result of acid hydrolysis at different pH levels (Figure 3b). The concerned linear regression coefficients of TAC on TPC and RSA were 0.9764 and 0.9695, respectively. This is also in accordance with the findings of Gomez-Plaza *et al*. who reported that TAC (FRAP) correlated much better with TPC than with anthocyanins (the latter showing insignificant correlation) for grape pomace [16]. Considering that both TPC and CUPRAC tests are based on an electron-transfer mechanism, high correlation of the phenolic content and antioxidant capacity of the colored vegetables was found by using the CUPRAC antioxidant capacity assay in several previous studies [6, 17, 18]. These results indicated that a wide variety of phenolics rather than anthocyanins alone play an important role in contributing to the whole TAC. The reason may be that anthocyanins in pomace could transform into other types of phenolics which may nearly maintain their antioxidant capacity during the acid hydrolysis [25, 26].

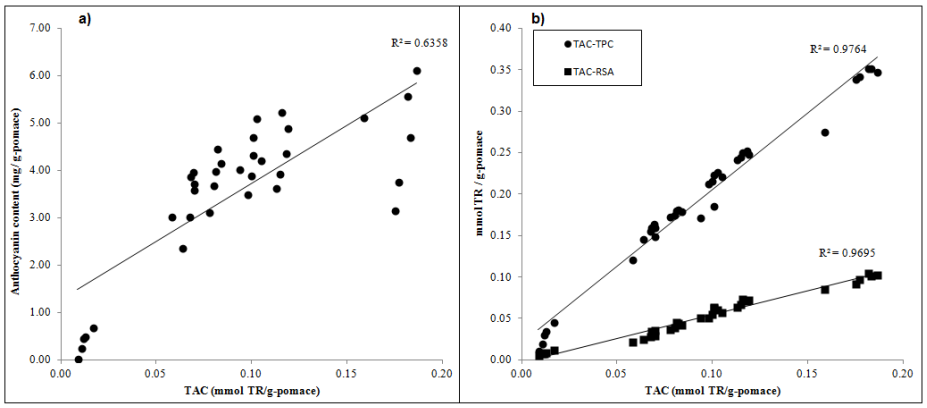

Figure 3. a) Correlation between the anthocyanin content and total antioxidant capacity (TAC), **b)** Correlation between total antioxidant capacity (TAC), total phenolic content (TPC) and free radical scavenging activity (RSA) of grape pomace as a result of acid hydrolysis in different pH treatment and control solutions.

Changes of anthocyanins and antioxidant of Concord grape pomace as a result of acid hydrolysis were investigated in this study. Optimal acid concentration and hydrolysis time were determined for releasing anthocyanins and other phenolics from grape pomace. Anthocyanin content, TPC, TAC, and RSA of pomace in acid hydrolysis were determined by multi test systems for different acid solutions. In addition, relationships of the anthocyanin content and other antioxidant properties of grape pomace during acid hydrolysis were investigated and discussed. The results of this study would be very useful for utilizing Concord grape pomace as an anthocyanin- and fiber-rich food ingredient or nutrition supplement. They could also provide guidance information for developing anthocyanin-rich red grape pomace cereal or bakery food products with highly retained anthocyanins.

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