# Determination Of Anthocyanin Content In Purple Corn Cob And Kernel And Development Of Efficient Separation Method

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## ABSTRACT

The color pigments present in purple corn cob and kernel were compared and the most efficient extraction method for anthocyanins was investigated without using enzymes (for processing cost reduction). The corn cob contained significantly higher concentration of color pigments than the kernel indicating that the cob is a potentially valuable by-product for color pigment market. Moreover, the processing cost would be lower because there is no starch is present in corn cob (removing the need of starch removal). Higher temperature resulted in a higher yield rate of anthocyanins but high temperature extraction of purple corn kernel showed some technical difficulties during filtration due to the presence of starch.


## INTRODUCTION

Purple corn (*Zea Mays*) is an emerging source of natural color due to its high anthocyanin content. Corn cobs are considered waste in the corn industry but purple corn cobs could be a valuable by-product due to the amount of anthocyanins. In this study, the concentration of anthocyanin in purple corn cob was compared to that in the kernel. Also, the method to extract maximum amount of anthocyanin with several different temperatures and filtration methods was evaluated to determine the efficiency of the processing with or without gelatinization.

## MATERIALS AND METHODS

Material and preparation:
Purple corn cob and kernel were finely ground using a lab-scale mill and than passed through a test sieve (0.0059 IN, USA standard test sieve, Fisher Scientific). Cob powder (1g) was brewed with hot water at 85°C for 10min while 1g of kernel powder was brewed at 25, 50, 60, and 85°C for 10 min. All cob and kernel extracts were divided into two groups for either centrifugation or filtration. For centrifugation, 45mL of infusion was centrifuged at 3500 r.p.m. for 30 min and remaining 45mL of infusion was filtered through Fisher Scientific Filter paper (25μm pore size).

Methods:
1. Total Phenolics (TP) (or total soluble phenolics) was determined using the Folin-Ciocalteau assay as early described by Swain and Hillis (1959) and expressed as gallic acid equivalent.





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2. Total liquor color was measured at 520nm using a Genesis 6 spectometer (Thermo Inc.). The absorbance of each purple corn infusion was recorded and the color value was determined.

3. Anthocyanins were tentatively identified and quantified using Agilent 1200 series HPLC. Their concentrations were expressed as cyanidin 3-glucoside equivalent. A gradient mobile phase consisted of Phase A (100% $H_2O$) and Phase B (60% Methanol and 40% $H_2O$) each adjusted to pH 2.4 using *o*-phosphoric acid. The gradient started by running 0 % Phase B for 1 min, 0-50 % Phase B over 10 min, 50-70 % Phase B in 10 min, 70-100 % Phase B in 20 min for a total run time of 40 min. The column was equilibrated with 100 % phase A for 2 min prior to the next sample injection. Anthocyanins were separated on a Dionex 250 x 4.6 mm Acclaim 120-C18 column run at 0.8 mL/min and detected by a UV-VIS detector at 520 nm.

4. Total anthocyanin content was measured using a spectrometric method. An aliquot of supernatant from each sample was properly diluted into a spectrometric linear range for anthocyanins (Abs 0.8 – 1.2). The proper dilution factor varied depending on the samples and the range was from 4 to 6. Two aliquots of 0.5mL of properly diluted stock solution with pH 3.0 citric acid buffer were added to test tubes containing 4.5mL of pH 1.0 and pH 4.5 buffers and they were thoroughly mixed by vortex for 10 sec. After staying at room temperature for 20 min, each solution was measured at 520nm and 700nm against blanks of pH 1.0 and 4.5 buffers. Total anthocyanin calculation was calculated by

Total anthocyanin (mg/L) = (A/a) X MW X 1000 X DF

Where: A = adjusted absorbance = $(A_{520}-A_{700})$buffer 1.0 – $(A_{520}-A_{700})$buffer 4.5, 1000 = molar to ppm, DF = dilution factor

Three buffers (pH 1.0, 3.0, and 4.5) were prepared as described in SEN-TN-0019.

## RESULTS AND DISCUSSION

Table 1 displays the differences of total anthocyanin, total liquor color, and two most prevalent anthocyanins such as cyanidin 3-glucoside and acylated cyanidin 3-glucoside in cob and kernel brewed at 85°C. As predicted, total anthocyanin, total concentration of cyanidin 3-glucoside and acylated cyanidin 3-glucoside, and total liquor color were significantly higher in purple corn cob than in kernel. Additionally, the cob infusion contained no starch compared to the kernel where high starch content interferes with the filtering process during corn kernel processing. When comparing anthocyanin extraction efficiency by centrifugation versus filtration, centrifuged extractions contained slightly or significantly higher amounts of color pigments. Due to pigment is absorbed into the filter paper during filtration, loss in anthocyanin might have occurred.

Table 2 illustrates the difference in anthocyanin content and color by different extraction temperatures. As the known temperature of corn starch gelatinization is between 62 ~ 72°C, 60°C has been applied in order to not cause gelatinization. Thus, it was expected that starch may have remained in the insoluble state at 60°C, but gelatinization visibly occurred at the temperature. Thus it is possible that gelatinization temperature for purple corn may be a little lower than regular purple corn. Since no gelatinization was observed at 50°C, the gelatinization temperature could be predicted between 51 and 60°C. For kernel extraction, the highest temperature was most effective for anthocyanins and resultant color. However, there was a significant loss of extraction yield because gelatinized starch interrupted the separation of




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anthocyanin and starch. It took 10 times longer to filter the extraction at 85°C than at 25 and 50°C. Also, when centrifuging, some water was absorbed into the gelatinized starch due to starch's swelling property. In the following study, an enzyme will be applied to kernel extraction to see the filtering efficiency when the structures of amylose and amylopectin were broken down.

The potential value of purple corn cob as a valuable by-product was evaluated. Since it is very inexpensive and contains significant amount of color pigment, the potential value for color pigment market is very high. Even though higher temperature extracts higher concentration of anthocyanin color pigment, it was not recommended in the separation processing due to the technical difficulty of extraction. The follow up study may be able to suggest that using enzyme could solve this problem.

Table 1. Differences of total anthocyanin, total liquor color, and two most prevalent anthocyanins such as cyanidin 3-glucoside and Acylated cyanidin 3-glucoside in cob and kernel brewed at 85°C.

| | **COB** | | **KERNEL** | |
|---|---|---|---|---|
| | **CEN[1]** | **FIL** | **CEN** | **FIL** |
| **Total Anthocyanin** | 15.44[2] | 14.21 | 3.89 | 3.34 |
| **Cyanidin 3-glucoside and Cyanidin 3-rutinoside** | 3.68 | 3.65 | 0.45 | 0.32 |
| **Total liquor color** | 3.04 | 3.04 | 1.34 | 1.60 |

1. Abbreviation used. CEN. Centrifuged, FIL. Filtered
2. Total anthocyanin and cyanidin 3-glucoside and cyanidin 3-rutinoside were expressed as g/L.

Table 2. Differences in total anthocyanin, total liquor color, and two most prevalent anthocyanins (cyanidin 3-glucoside and Acylated cyanidin 3-glucoside) in purple corn kernel infusion extracted at 25, 50, 60, and 85°C.

| Temp | Separation | Total Anthocyanin (g/L) | Cyanidin glycosides (mg/L) | Liquor color (no unit) |
|---|---|---|---|---|
| 25°C | CEN[1] | 3.09 | 101.1 | 0.89 |
| | FIL | 2.90 | 95.1 | 0.76 |
| 50°C | CEN | 3.21 | 178.4 | 0.90 |
| | FIL | 2.90 | 129.9 | 0.85 |
| 60°C | CEN | 3.03 | 149.0 | 0.82 |
| | FIL | 2.90 | 144.1 | 0.81 |
| 85°C | CEN | 3.89 | 450.1 | 1.34 |
| | FIL | 3.34 | 324.1 | 1.60 |

1. Abbreviation used. CEN. Centrifuged, FIL. Filtered

## REFERENCE CITED

Swain, T.; Hillis, W. E. The phenolic constituents of *Purmus domestica*. I. The quantitative analysis of phenolic constituents. *J. Sci. Food. Agric*. **1959**, *10*, 63-68.





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