



# **Maximum Retention Of Anthocyanins In Purple Corn Kernel During Processing**

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**Youngmok Kim, Ph.D. and Daniel J. Wampler, Ph.D.**

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## **ABSTRACT**

Even though purple corn is a significant source of anthocyanin, it is not widely available in the US due to its limited growing region. Since anthocyanin is a very unstable compound being sensitive to light, high temperature, extreme pH, and oxidation, the processing method should be carefully chosen for the maximum extraction and minimum loss of anthocyanin. In the present study, a method to maintain maximum anthocyanin levels during extraction from purple corn was developed.

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## **INTRODUCTION**

Purple corn (*Zea Mays*) is known as a significant source of natural colorant due to high anthocyanin concentration. However, corn (especially the kernel) contains a high concentration of starch which is undesirable during color pigment extraction, so removal of starch must be preceded for maximum color extraction. In the present study, methods to retain maximum amount of anthocyanins during the removal of starch from purple corn kernels were developed.

## **MATERIALS AND METHODS**

Raw Material: Purple corn (*Maize morato*. Inca Purple corn. Amazonas Imports, Inc. Sun Valley, CA).

Sample treatment: Purple corn was finely ground using a lab-scale mill and 5g of fine powder was brewed with 100mL hot water (90°C) for 10 min. The infusion was equally divided into five groups and the first three groups were filtered through p-8-creped filter paper when the infusion was still hot. The fourth and fifth groups were remained unfiltered. The description of 5 groups was illustrated in Table 1. Chemical analyses were conducted after 24 hours of incubation.

Table 1. Treatments of hot water brewed purple corn infusions.

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7255 Hamilton Enterprise Park Drive  
Hamilton, OH 45011  
Tel: 513-892-7100  
www.sensusflavors.com

No.	Treatment
1	Brewed – filtering – stay at room temp
2	Brewed – filtering – lowering pH to 3 w/10% citric acid solution – stay at room temp
3	Brewed – filtering – stay at refrigerated temp
4	Brewed – stay at room temp
5	Brewed – immediately centrifuged – stay at room temp

#### Analytical Methods:

1. 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<sub>2</sub>O) and Phase B (60 % Methanol and 40% H<sub>2</sub>O) 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.

2. Total liquor color was measured at 520nm using Genesis 6 spectrometer (Thermo Inc.). The absorbance of each tea infusion was recorded and the color value was calculated.

3. Total anthocyanin content was measured using a spectrometric method. An aliquot 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 using a vortex mixer for 10 sec. After staying for 20min at room temperature, each solution was measured at 520nm and 700nm against blanks of pH 1.0 and 4.5 buffers. Total anthocyanin calculation was done based on Beer's Law,

Beer's Law:  $A = abc$

Where: A = absorbance, a = molar extinction coefficient (29,600 for cyanidin 3-glucoside – MW = 457.16), b = lightpath (1 cm), c = concentration.

Therefore, total concentration was calculated by

Total anthocyanin (mg/L) =  $(A/a) \times MW \times 1000 \times DF$

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

Three buffers (pH 1.0, 3.0, and 4.5) were made by following methods:

(1)pH 3.0 citric acid buffer (1 L):

1. Add 19.24g of anhydrous citric acid to a 1L flask and dissolve in ~800mL water.
2. Add 1.91g of NaOH pellets and dissolve.
3. Adjust volume to ~950 mL and adjust pH to exactly 3.00 and bring to volume.

(2)pH 1.0 Buffer (1 L):

1. Add 62.5mL of concentrated HCl to 687.5 mL water (750mL total volume).

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2. Dissolve 3.72g KCl in 250mL water.
3. Combine the two solutions and adjust pH to 1.0 with 1M HCl

(3)pH 4.5 Buffer (1 L):

1. Add 236.4 mL of 1 M HCl (19.7mL concentrated HCl to 216.7 mL water) to 54.44g sodium acetate (trihydrate).
2. Adjust to pH 4.5 with either 1 M HCl or sodium acetate.

## **RESULTS AND DISCUSSION**

As shown in Table 2, total anthocyanin (TA) was significantly higher in no.1, 2, and 3 samples compared to no 4 and 5 (non-filtered samples) (Table 2). Most of gelatinized starch was removed from 1, 2, and 3 by filtering but not from no.4 and 5. Thus, it is hypothesized that remaining starch in test tubes caused detrimental effects on the anthocyanin content in the purple corn extract. During 24 hr incubation, retrograded starch became insoluble and may bind to anthocyanin compounds which generated precipitation. Among filtered samples, TA was highest in no.2 sample (acidified). Anthocyanin was highly unstable at high pH, lowering the pH would be a key to maintain maximum level of anthocyanin during storage as many scientific researches have proven that anthocyanin is more stable in acidic environment. It was predicted that the sample stored at refrigerated temperature contained higher concentration of anthocyanin due to higher stability of anthocyanin at cold temperature, but only insignificant difference was observed in this study. The color strength of each sample was highest in no.2 sample as observed in TA content. HPLC data strongly support the current observation (Figure 1). Total concentration of two major anthocyanins in no.2 sample was higher by 23 and 32% than in no. 1 and 3 samples, respectively (Figure 2).

Besides anthocyanin content, no starch residue was found in no.2 sample while others showed starch precipitate regardless of filtering (Figure 3). Since starch was gelatinized by hot water extraction, solubilized starch granules passed through filter paper. As the extracts cooled down, dissolved starch became insoluble via retrogradation. In Figure 4, starch precipitate could be observed in all purple corn extracts except no.2. This was because the addition of acid may cause weak acid hydrolysis resulting in break down of starch structure in no.2 sample.

The optimum extraction and storage conditions for purple corn were investigated.

1. Hot water extraction
2. Immediate filtering or centrifugation (centrifugation is recommended due to loss of anthocyanin pigment by filtering – dying filter paper)
3. Acidification with organic acid (e.g. citric acid solution)
4. Storage at cold temperature is recommended

Table 2. Total anthocyanin (TA) and total liquid color (TC) in purple corn extract after removal of starch.

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	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
TA (mg/L)	92.67	96.53	90.35	78.77	60.23
TC	1.04	2.77	1.30	1.54	0.96

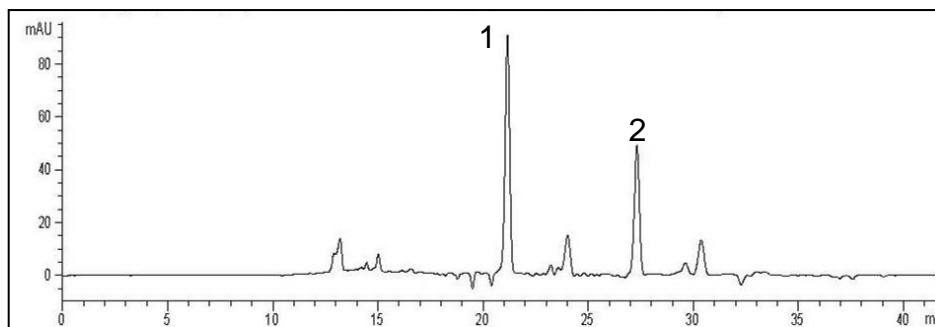


Figure 1. HPLC chromatogram of purple corn extract. Peaks were tentatively identified based on the similarity of spectra. two major anthocyanins. 1. Cyanidin 3-glucoside, 2. Acylated cyanidin 3-glucoside.

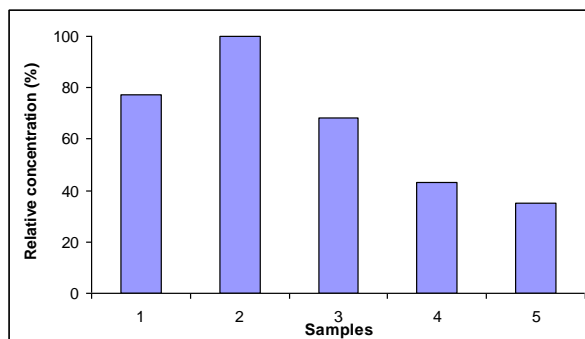


Figure 2. Relative concentrations of two major anthocyanin compounds in purple corn.



Figure 3. Starch precipitation in each sample after centrifugation.