Elderberry Flower Production and Cyanide Concern

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American Elderberry

organic drinks
100% natural

Elderberry Flower Production

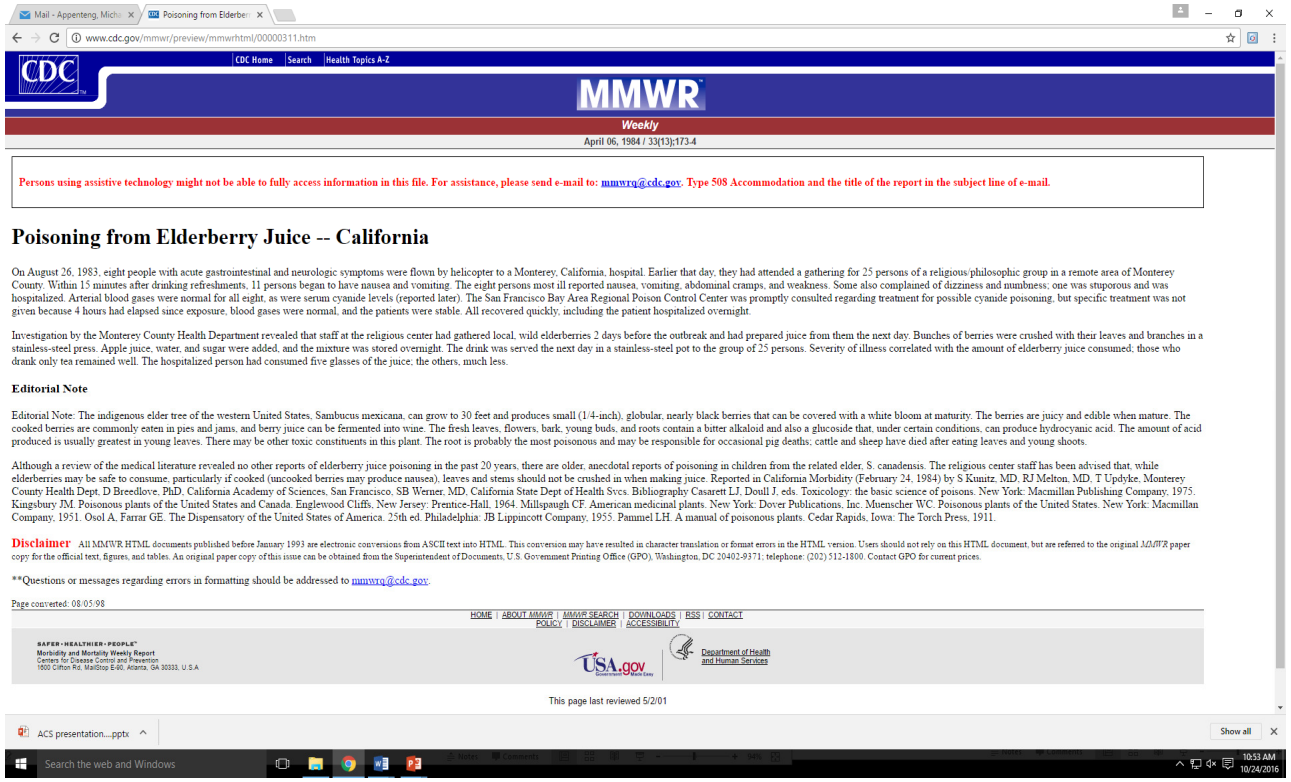
0, 25, 50 and 100% flower harvest

Flower productivity
Effect on remaining fruit yield, size, and quality
Effect on plant growth

SORRY – Stay Tuned!

CDC report - Poisoning incident

- California, August 26, 1983



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MMWR

Weekly

April 06, 1984 / 33(13);173-4

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Poisoning from Elderberry Juice -- California

On August 26, 1983, eight people with acute gastrointestinal and neurologic symptoms were flown by helicopter to a Monterey, California, hospital. Earlier that day, they had attended a gathering for 25 persons of a religious/philosophic group in a remote area of Monterey County. Within 15 minutes after drinking refreshments, 11 persons began to have nausea and vomiting. The eight persons most ill reported nausea, vomiting, abdominal cramps, and weakness. Some also complained of dizziness and numbness; one was stuporous and was hospitalized. Arterial blood gases were normal for all eight, as were serum cyanide levels (reported later). The San Francisco Bay Area Regional Poison Control Center was promptly consulted regarding treatment for possible cyanide poisoning, but specific treatment was not given because 4 hours had elapsed since exposure, blood gases were normal, and the patients were stable. All recovered quickly, including the patient hospitalized overnight.

Investigation by the Monterey County Health Department revealed that staff at the religious center had gathered local, wild elderberries 2 days before the outbreak and had prepared juice from them the next day. Bunches of berries were crushed with their leaves and branches in a stainless-steel press. Apple juice, water, and sugar were added, and the mixture was stored overnight. The drink was served the next day in a stainless-steel pot to the group of 25 persons. Severity of illness correlated with the amount of elderberry juice consumed; those who drank only tea remained well. The hospitalized person had consumed five glasses of the juice; the others, much less.

Editorial Note

Editorial Note: The indigenous elder tree of the western United States, Sambucus mexicana, can grow to 30 feet and produces small (1/4-inch), globular, nearly black berries that can be covered with a white bloom at maturity. The berries are juicy and edible when mature. The cooked berries are commonly eaten in pies and jams, and berry juice can be fermented into wine. The fresh leaves, flowers, bark, young buds, and roots contain a bitter alkaloid and also a glucoside that, under certain conditions, can produce hydrocyanic acid. The amount of acid produced is usually greatest in young leaves. There may be other toxic constituents in this plant. The root is probably the most poisonous and may be responsible for occasional pig deaths; cattle and sheep have died after eating leaves and young shoots.

Although a review of the medical literature revealed no other reports of elderberry juice poisoning in the past 20 years, there are older, anecdotal reports of poisoning in children from the related elder, S. canadensis. The religious center staff has been advised that, while elderberries may be safe to consume, particularly if cooked (uncooked berries may produce nausea), leaves and stems should not be crushed in when making juice. Reported in California Morbidity (February 24, 1984) by S Kunitz, MD, RJ Melton, MD, T Updyke, Monterey County Health Dept, D Breedlove, PhD, California Academy of Sciences, San Francisco, SB Werner, MD, California State Dept of Health Svcs. Bibliography Casarett LJ, Doull J, eds. Toxicology: the basic science of poisons. New York: Macmillan Publishing Company, 1975. Kingsbury JM. Poisonous plants of the United States and Canada. Englewood Cliffs, New Jersey: Prentice-Hall, 1964. Millspaugh CF. American medicinal plants. New York: Dover Publications, Inc. Muenscher WC. Poisonous plants of the United States. New York: Macmillan Company, 1951. Osol A, Farrar GE. The Dispensatory of the United States of America. 25th ed. Philadelphia: JB Lippincott Company, 1955. Pammel LH. A manual of poisonous plants. Cedar Rapids, Iowa: The Torch Press, 1911.

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- Uncertainty regarding the presence of cyanogenic glycosides (CNG's) in American Elderberry.

Cyanogenic Glycosides (CNG's)

- Nitrogenous secondary plant metabolites

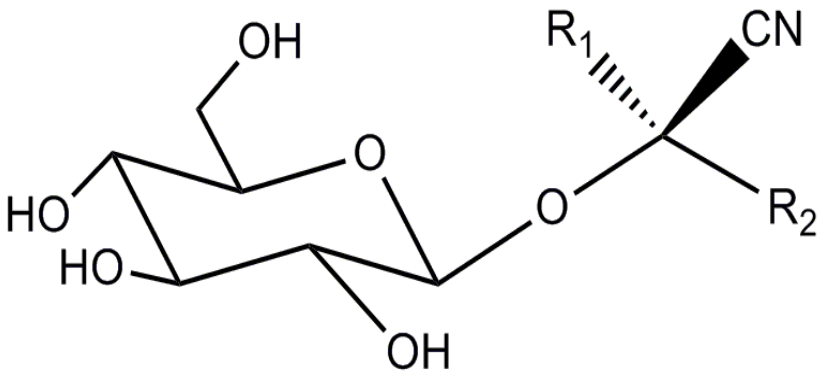


Figure 1: Generic structure of CNG's

- about 60 known compounds
- Found in over 2600 Plant species.
- Plant chemical defense compounds
- Subacute and acute cyanide poisoning depending on dose.
- Threshold to lethal dose (0.5 to 3.5 mg per kg of body weight).

Research Objective

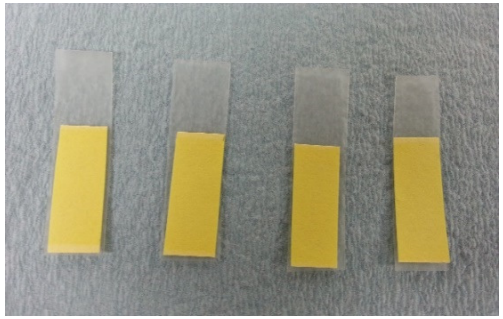
The main objective of this research to accurately analyze (identify and quantify) CNG's in American elderberry.

- Method of analysis

  ~ Picrate Paper Method

  ~ LC-MS/MS Method

Materials & Method



Picrate Paper- filter paper in Picrate solution (1.4% w/v picric acid in 50 mL of 2.5% w/v Na_2CO_3

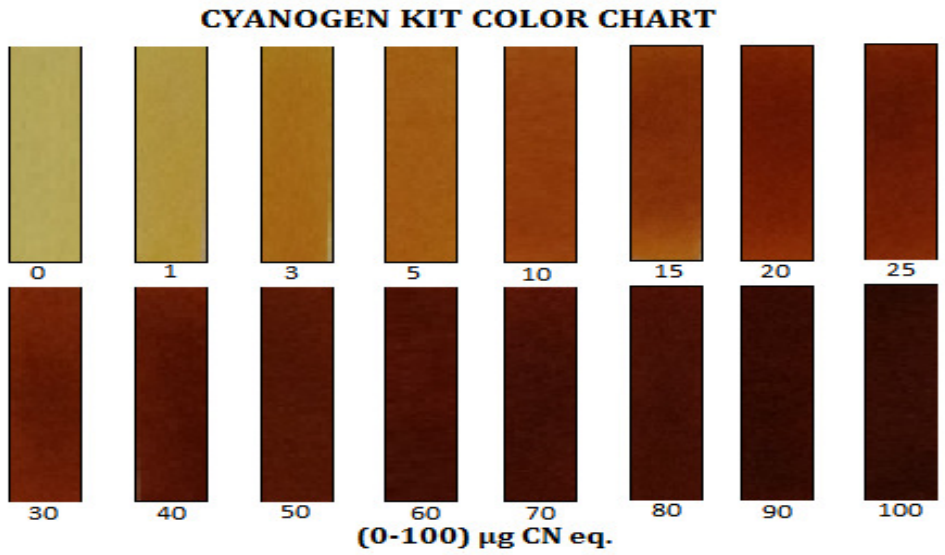
1

Amygdalin (0-100µg) + 50µL of 3U/mL β-glucosidase enzymes in pH 8 phosphate buffer + picrate paper

2

Overnight heating (16-24hrs/ 30-40°C)

3



Control Test - Apple seeds

Two apple varieties: Granny Smith (GS) and Gala (G) apple seeds and juice

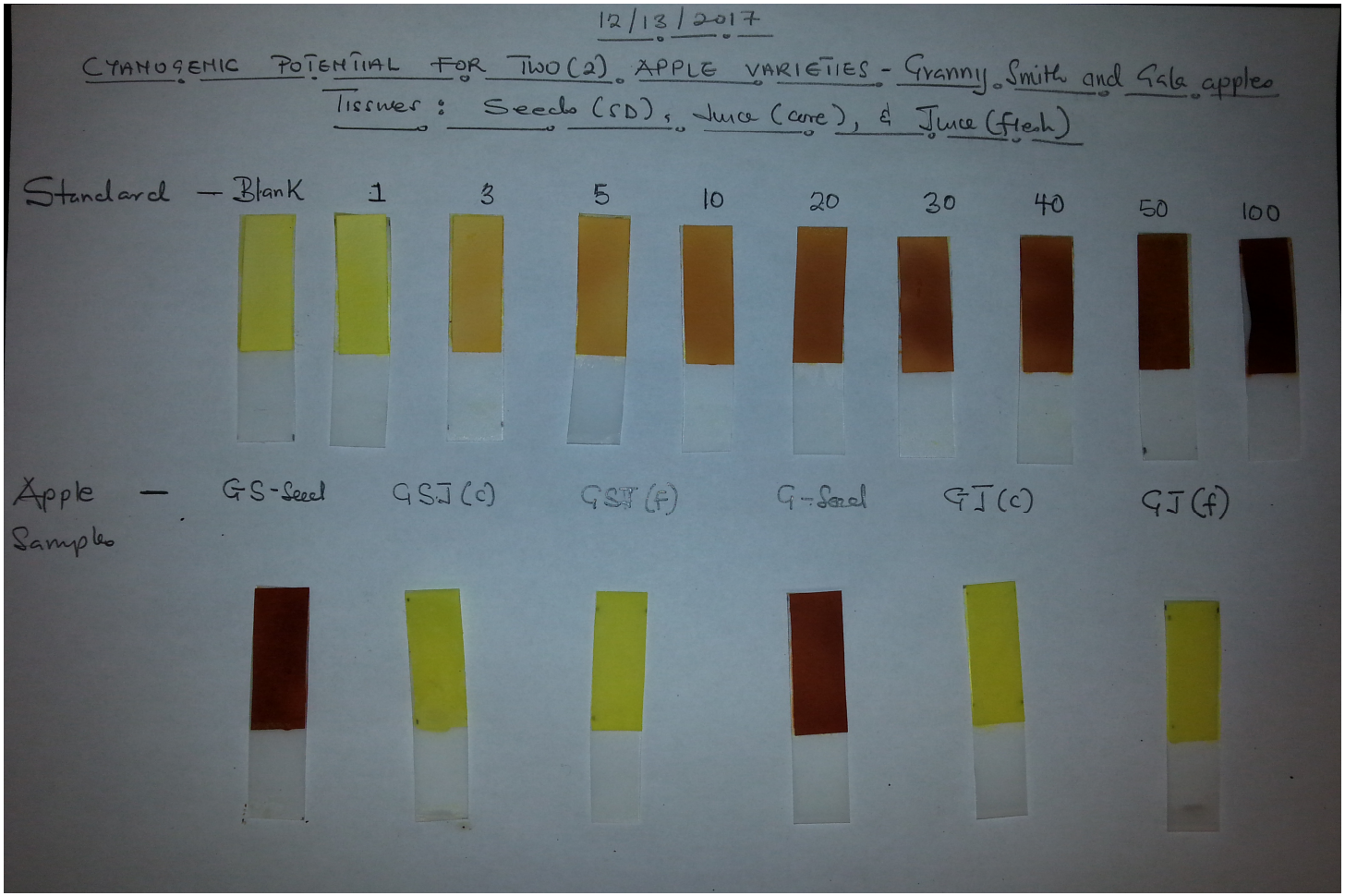


Figure 5: Picrate Paper results showing visible color change for apple seeds

Control Test - Apple seeds

Two apple varieties: Granny Smith (GS) and Gala (G) apple seeds and juice

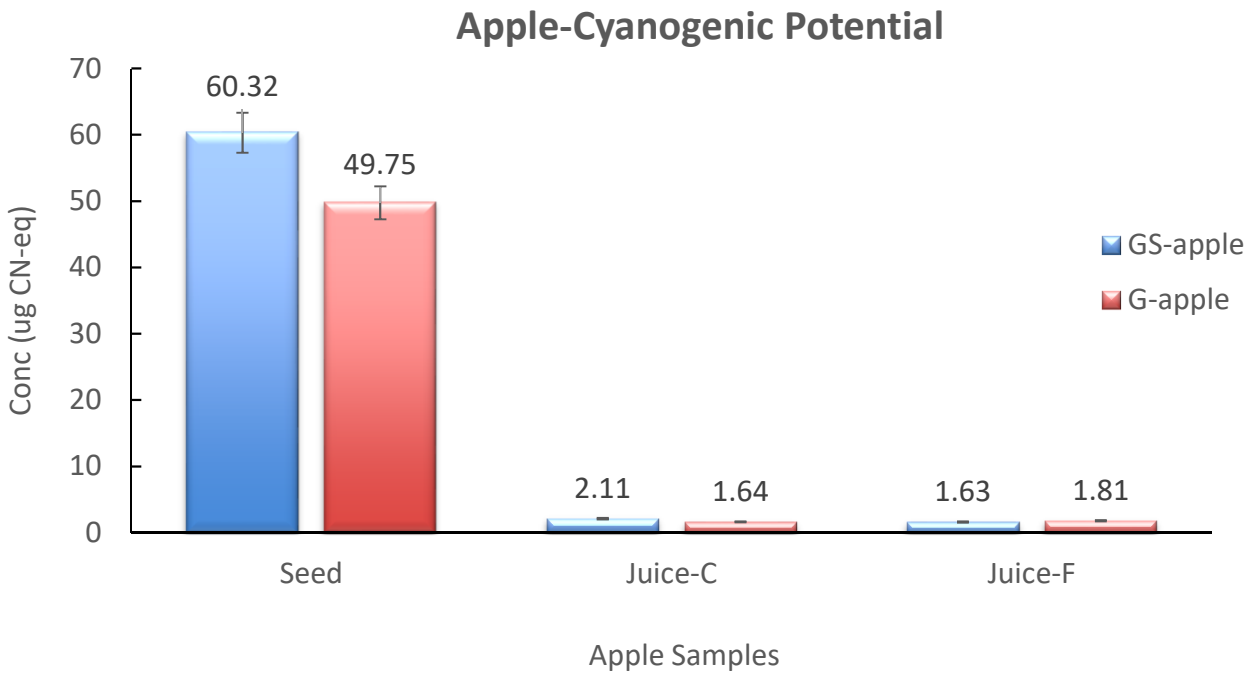


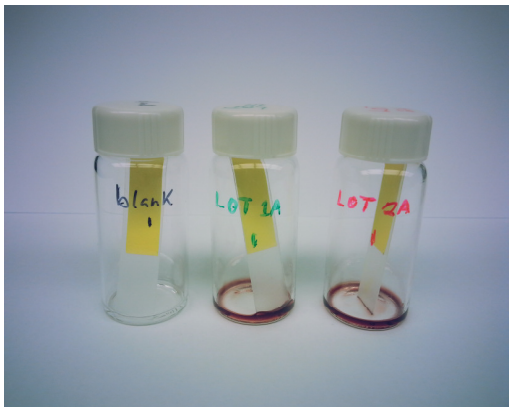
Figure 6: Picrate Paper results showing results for apple seeds and juice

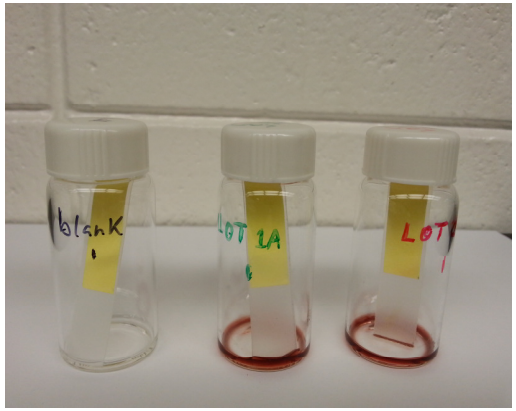
Results: Visible color change. Very high traces of cyanide detected.
UV-Vis analysis at 510nm (λ): Avg. concentration between (49.75-60.32) µg CN^- eq. per 0.1g or mL of sample
Levels: Highest in the seeds of both varieties.

Sample Test 1– Commercial Juice Sample

Results: No color change. No cyanide detected

UV-Vis analysis at 510nm (λ): Concentration < 0.143µg CN^- eq. (LLOQ)





100µL Juice + 10µL of 3U/mL β-glucosidase enzymes in pH 8 phosphate buffer + picrate paper

Before

No Color Change

After

Sample Test 2– Ozone & Ozark AE Samples

Sample tissues- juice, seeds, skin, stem

Figure 6: Picrate Paper results for tissues of Ozone and Ozark elderberry samples

Sample Test 2– Ozone & Ozark AE Samples

Sample tissues- juice, seeds, skin, stem

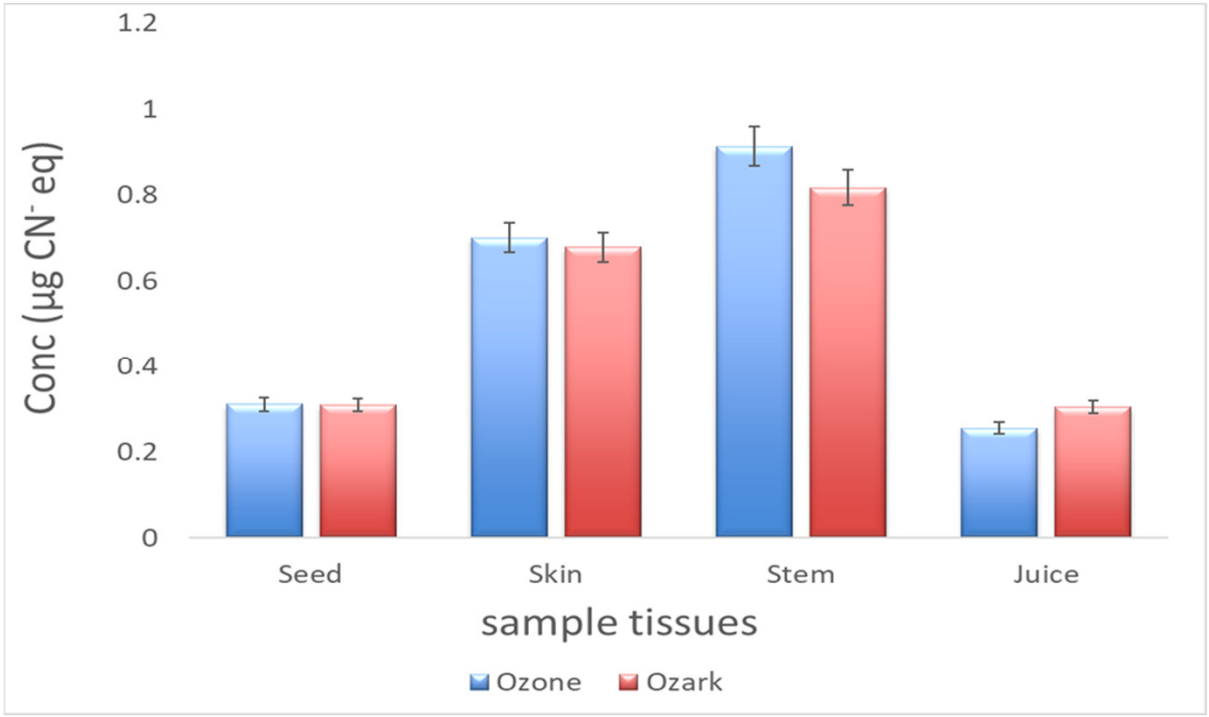


Figure 6: Total Cyanogenic potential for different tissues of Ozone and Ozark elderberry

Results: No visible color change. Very low levels of cyanide detected.
UV-Vis analysis at 510nm (λ): Avg. concentration < 1 µg (0.26-0.92) µg CN^- eq. per 0.1g or mL of sample
Concentration: Highest in Stems and lowest in Juice

Sample Test 3–Composite AE samples

Composite AE Samples: Ococee, Bob Gordon, Ozark, York & Wyldewood

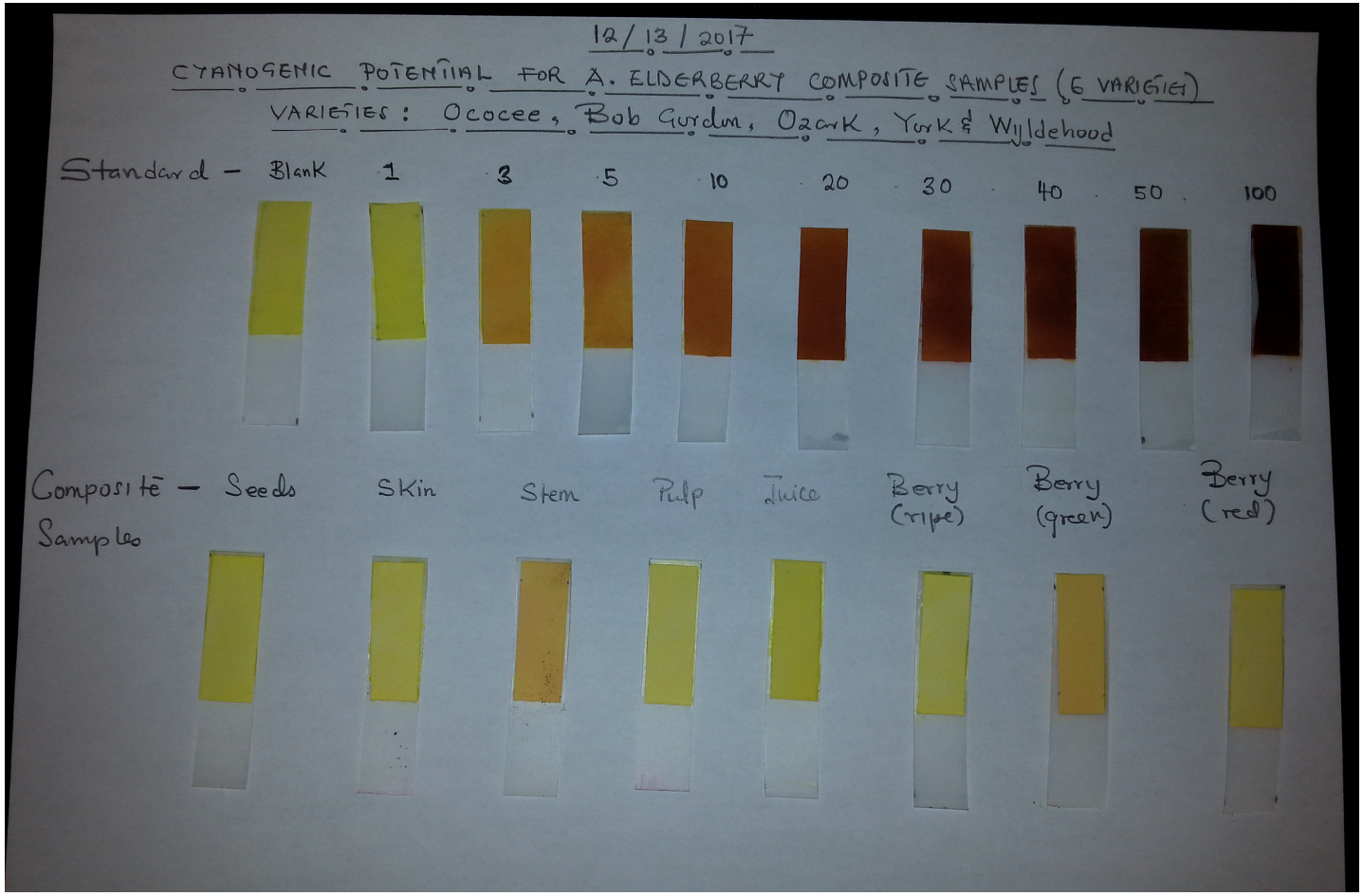


Figure 6: Picrate Paper results for tissues of composite elderberry samples

Sample Test 3–Composite AE samples

Composite AE Samples: Ococee, Bob Gordon, Ozark, York & Wyldewood

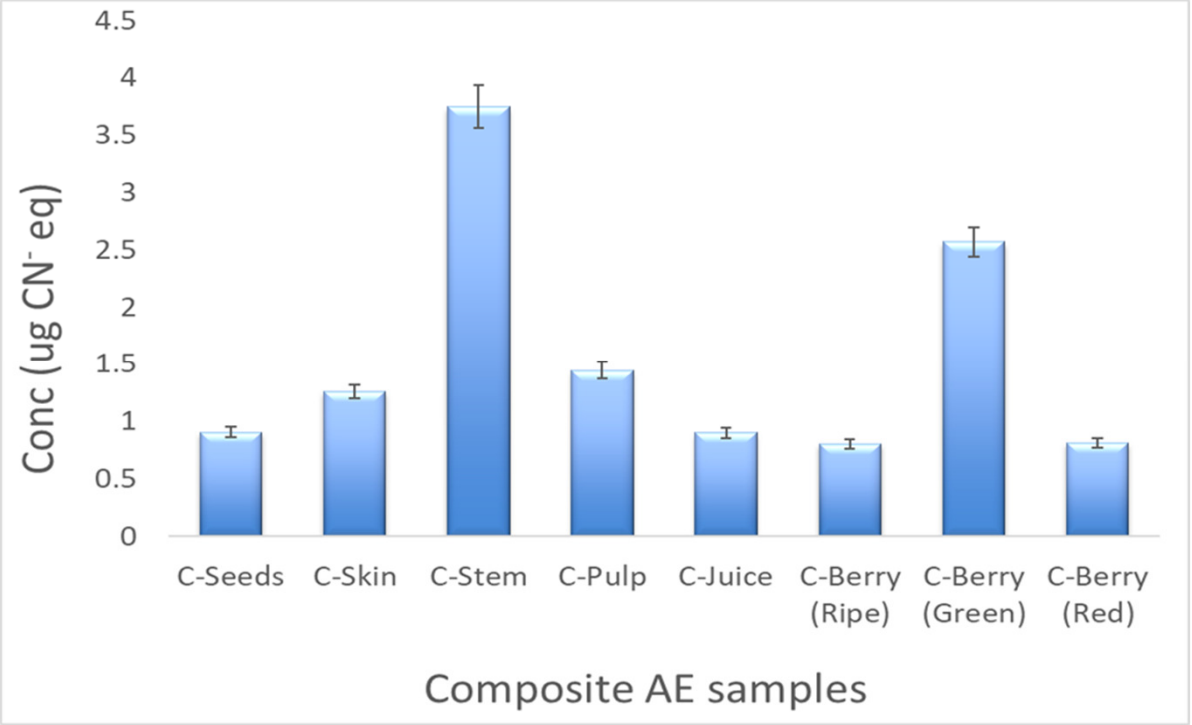


Figure 6: Total Cyanogenic potential for different tissues of composite elderberry samples

Results: Visible color change. Very low levels of cyanide detected.
UV-Vis analysis at 510nm (λ): Avg. concentration < 4 µg (0.80-3.74) µg CN^- eq. per 0.1g or mL of sample
Levels: Highest in stems & green berries. Lowest in juice.

Results – compared to apple studies

Acute CN toxicity
0.5 - 3.5 mg/kg of body weight

Table 1: Comparing results for this work (AE) to apple studies (15 varieties)

Elderberry samples		Concentration ± standard deviation (µg/g)			
		Amygdalin	**Dhurrin**	**Prunasin**	**Linamarin**
Seeds	Ozone	2.38 ± 0.09	0.27 ± 0.05	0.58 ± 0.04	0.12 ± 0.06
	Ozark	0.68 ± 0.12	0.22 ± 0.03	0.36 ± 0.05	0.13 ± 0.05
Juice	Ozone	1.57 ± 0.08	0.70 ± 0.12	1.45 ± 0.06	0.29 ± 0.03
	Ozark	0.36 ± 0.03	0.63 ± 0.04	2.36 ± 0.08	0.31 ± 0.01
Skin	Ozone	6.38 ± 0.40	0.12 ± 0.08	2.39 ± 0.04	0.75 ± 0.06
	Ozark	3.48 ± 0.14	1.46 ± 0.20	2.53 ± 0.08	0.90 ± 0.11
Stem	Ozone	5.42 ± 0.12	0.94 ± 0.06	2.84 ± 0.02	0.48 ± 0.04
	Ozark	2.15 ± 0.17	1.91 ± 0.03	3.07 ± 0.06	0.57 ± 0.06
Apple[3]	Seeds	**1000 – 4000**			
	Pressed Juice	**10 – 40**			
	Commercial Juice	**1-7**			

SUMMARY-(AE-µg/g) **stems** (0.48-5.42), **skin** (0.12-6.38), **seeds** (0.12-2.38;) & **juice** (0.31-2.36) **(This work)**

Results – compared to other studies

Acute CN toxicity

0.5 - 3.5 mg/kg of body weight

Table 2: Comparing results for this work with American Elderberry (AE) to other studies using European Elderberry (EE)

Elderberry	Tissue Type	CNG's (µg/g)	Reference
American	Pressed juice	0.31 - 2.36	This work
	All tissues	0.12 - 6.38	This work
European	Pressed juice	18.8 ± 4.3	
	Processed Juice	10.6 ± 0.7	
	Tea	3.8 ± 1.7	
	Spread	0.8 ± 0.19	
	liqueur	0.8 ± 0.21	
	flowers	1.23 - 18.88	
	Leaves	27.68 - 209.61	

Processing methods

- Effectiveness

  ~ *Montagnac et.al.*[12]-processing steps, the sequence utilized & time-dependent.
  e.g. soaking, fermenting and roasting removes about 98% CNG's

  ~ *Senica et.al.*[13]-thermal processing, time and type of extraction solution

Table 3: Effect of thermal processing on CNG'S levels

Elderberry	CNG's reduction%
Juice	44
Tea	80
Liquor	96
Spread	96

12. Sen Montagnac J. A., Davis C. R., Tanumihardjo S. A. *Compr. Rev. Food Sci. Food Safety* **2009**, 8, 17–27.
13. Senica, M., Stampar, F., Veberic, R., & Mikulic-Petkovsek, M. (2016). *Food Science and Technology* **2016**, 72, 182-188

Cyanogenesis

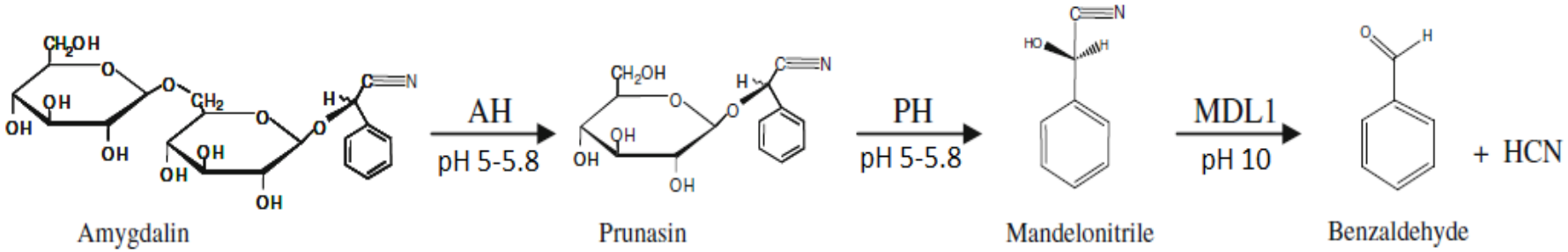


Figure 7: Enzymatic breakdown of amygdalin (a CNG) by endogenous enzymes: amygdalin hydrolase (AH), prunasin hydrolase (PH), mandelonitrile lyase 1(MDL1)

5. Islamiyat F. Bolarinwa, Caroline Orfila, Michael R.A. Morgan (**2014** *Food Chemistry* 152, 133–139

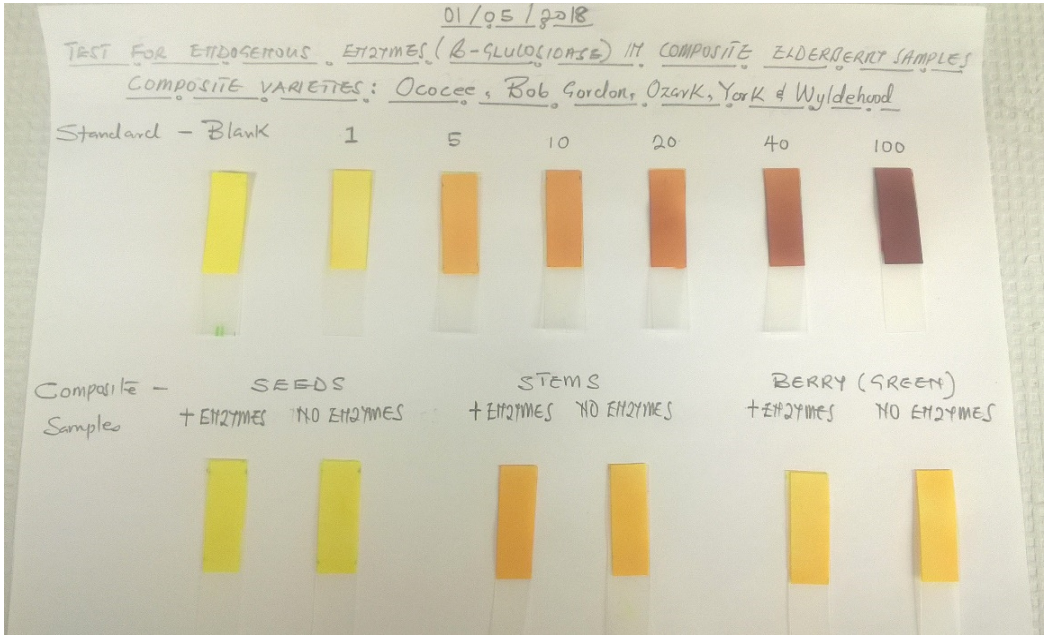
Test for endogenous enzymes

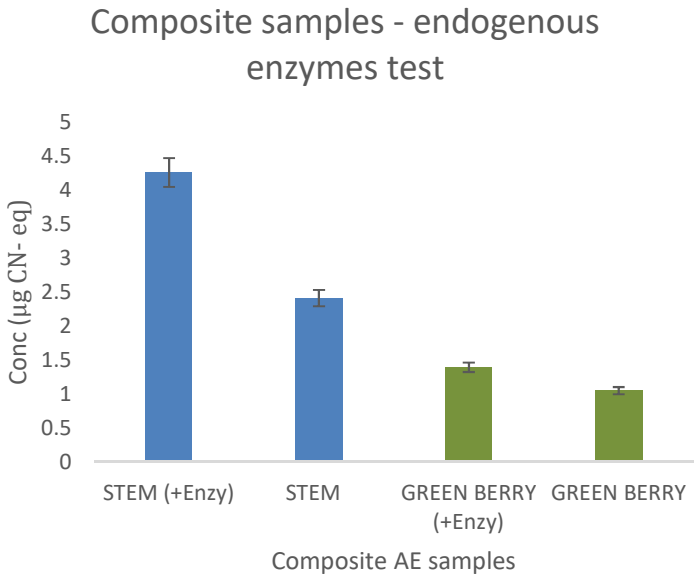
Composite elderberry tissues: Stems and green berry

Sep up: with and without external enzymes

Results: Lower CN- released (55 – 75%) in tissues without external enzymes.

Deductions: Although the elderberry tissues (stems and green berry) contain appreciable amount of endogenous beta glucosidase enzymes, it may not be sufficient for complete hydrolysis of all cyanogenic glycosides when the tissues are disrupted





Summary

- Picrate paper method was successfully used to assess the total cyanogenic potential.
- A control test with two apple varieties showed high levels of cyanide in the seeds.
- No cyanide was detected in commercial (processed) elderberry juice
- Levels of cyanide detected in tissues of fresh berries were very low; lowest in juice & seeds and highest in stems & green berries.
- Levels of CNG's detected with LC-MS method were very low in all tissues and consistent with picrate results.
- Detected levels pose no threat to American Elderberry consumers. Excluding stems, green berries and leaves in juice preparation is recommended.