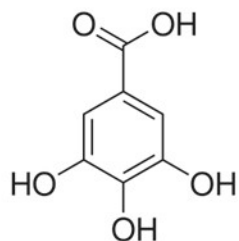
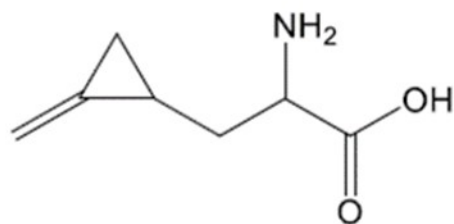


# Compounds to be Analyzed

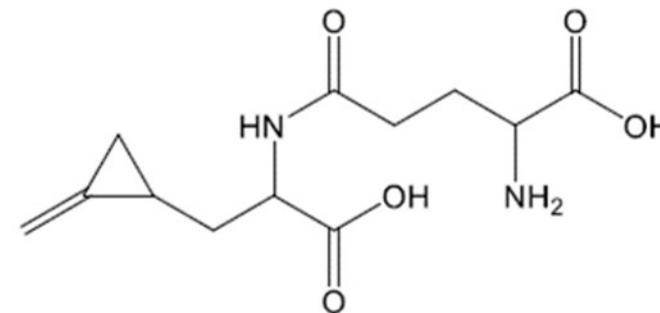
Absolute quantification of gallic acid and ellagic acid (both free and hydrolyzed)  
Relative quantification of four amino acids, HPA, HPB, MCPPrG, and Glutamyl-MCPrG



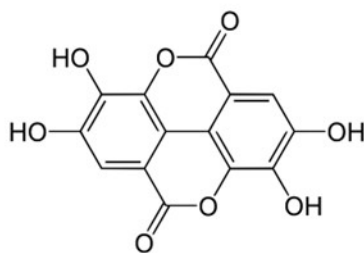
Gallic acid, C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>  
Exact Mass 170.0215



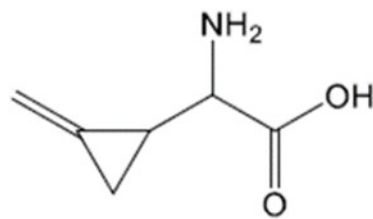
Hypoglycin A, C<sub>7</sub>H<sub>11</sub>NO<sub>2</sub>  
Exact Mass 141.0790



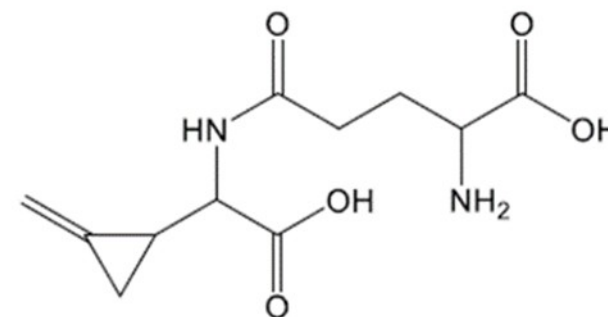
Hypoglycin B (Glutamyl Hypoglycin A)  
C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>, Exact Mass 270.1215



Ellagic acid, C<sub>14</sub>H<sub>6</sub>O<sub>8</sub>  
Exact Mass 302.0062



Methylene cyclopropylglycine (MCPrG)  
C<sub>6</sub>H<sub>9</sub>NO<sub>2</sub>, Exact Mass 127.0633



Glutamyl (MCPrG) C<sub>6</sub>H<sub>9</sub>NO<sub>2</sub>  
Exact Mass 256.1059

# Experimental

## 1. Sample preparation:

For the analysis of gallic acid, HGA, HGB, MCPPrG, and glutamyl MCPPrG, 10 mg of homogenized samples were accurately weighed and extracted using 1 mL of 5% methanol [1]. The extraction process involved treating the samples with methanol, followed by 10 minutes of sonication, 1 hour of shaking, centrifugation and collection of aliquots for injection into the UHPLC-qTOF-MS. Calibration standards for gallic acid were prepared at concentrations of 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, and 2.9062  $\mu\text{g}/\text{mL}$  in 5% methanol.

For ellagic acid analysis, 10 mg of each homogenized and weighed sample was extracted using 1mL 100% ethanol, [2] following the same extraction steps as described above, and prepared for UHPLC-qTOF-MS injection. Hydrolyzed ellagitannins were analyzed by performing acid hydrolysis on separately weighed 10 mg samples, which were treated with 4 M HCl and incubated at 90°C for 24 hours. Aliquots were collected, the pH adjusted to 2.5 and filtered using a 0.20  $\mu\text{m}$  PVDF filter before injection. Pellets remaining after the initial hydrolysis step were further extracted using a 50:50 DMSO-methanol mixture, following the same extraction procedure, and prepared for injection into the UHPLC-qTOF-MS. The calibration standards for ellagic acid were prepared at concentrations of 620, 310, 155, 77.5, 38.75, 19.375, 9.6875, and 4.8438  $\mu\text{g}/\text{mL}$  in 100% methanol.

## 2. Analysis using UHPLC-qTOF-MS

The samples prepared for injection were analyzed in a Waters Acquity UHPLC system equipped with a photodiode array detector and an Bruker Impact II qTOF mass spectrometer detector. Chromatographic separation was performed on a reverse phase BEH C18 column (100 mm × 2.1 mm, 1.7 μm particle size) (Waters). The mobile phases were water with 0.1% formic acid (A) and acetonitrile (B) following a gradient profile of 0–20 min, 5–17% B. A volume of 2 μL of sample was injected onto the column operating at 60°C at a flow rate of 0.560 mL/min. The qTOF mass spectrometer was equipped with an electrospray interface (ESI). Nitrogen was used as drying gas with a flow of 10 L/min at a temperature of 250 °C and nebulizing gas at pressure of 40 psi. The capillary voltage was set at 4.5 kV. The samples were analyzed in positive ion mode in mass spectrometer.

The data analysis and quantification was performed using TASQ (Bruker) software. The parameters used for the data analysis is as following:

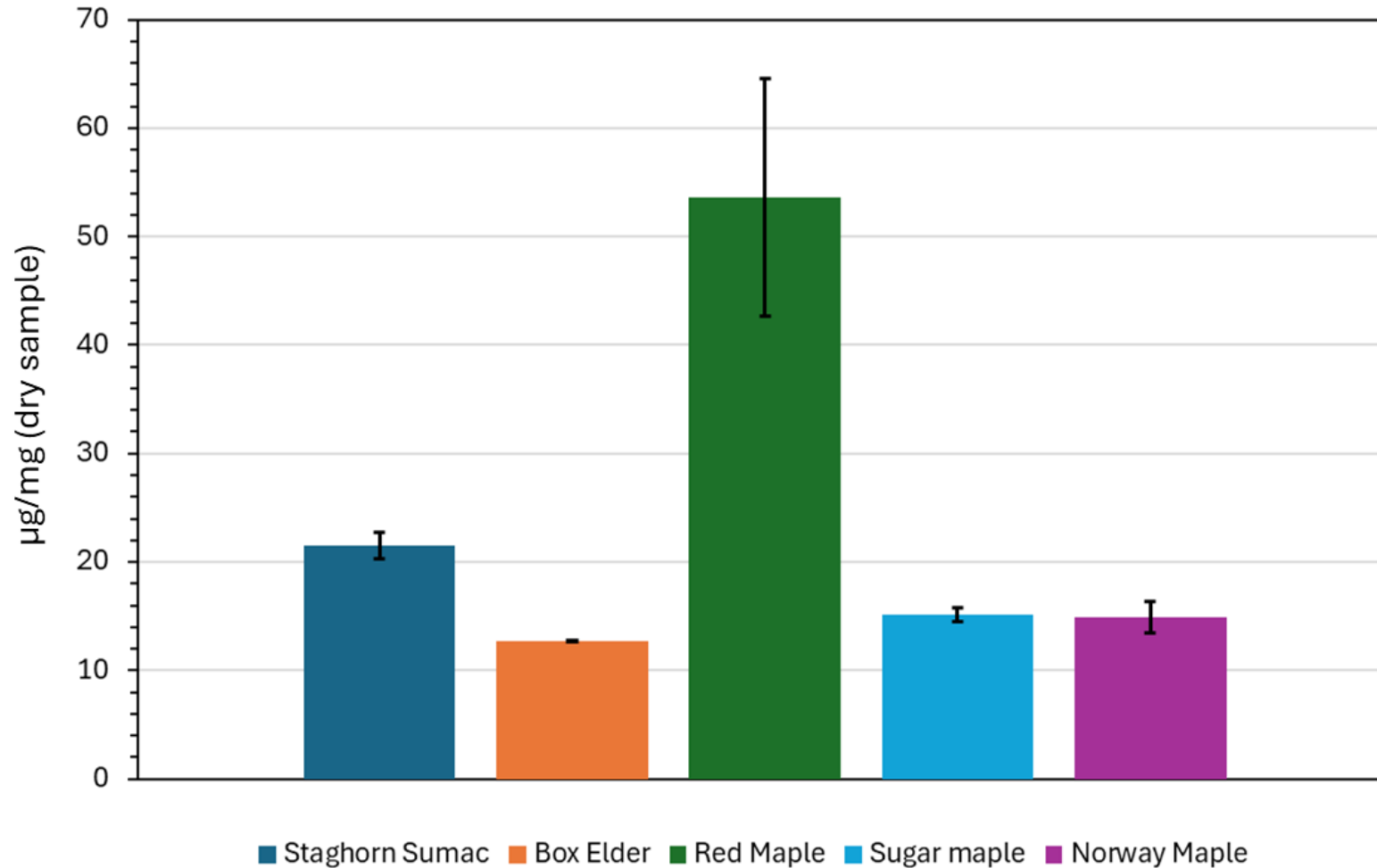
Ellagic acid: (RT 7.70min, [M+H]<sup>+</sup> =303.0140) Gallic Acid (RT 0.88min, [M+H]<sup>+</sup> =171.0290), HGA (RT 0.86min, [M+H]<sup>+</sup> =142.0868) , HGB (RT 2.12min, [M+H]<sup>+</sup> =271.1294), MCPPrG (RT 0.57min, [M+H]<sup>+</sup> =128.0712), Glutamyl MCPPrG (RT 1.15min, [M+H]<sup>+</sup> =257.1137).

1. *Toxins* **2022**, 14, 608. <https://doi.org/10.3390/toxins14090608>

2. *Reviews in Analytical Chemistry* 2020; 39: 31–44

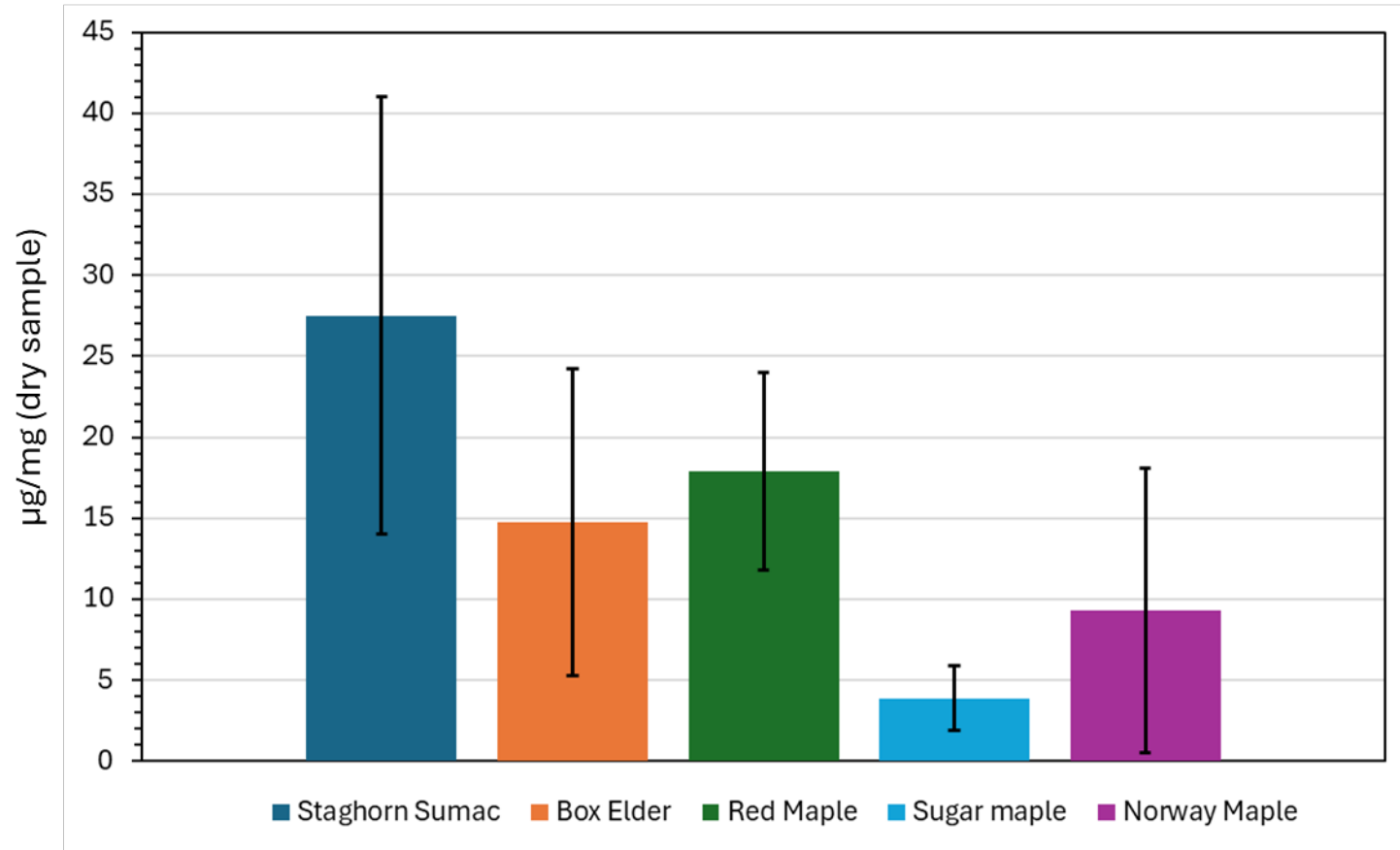
3. *J. Agric. Food Chem.* 2015, 63, 6555–6566.DOI: 10.1021/acs.jafc.5b02062

# Average Free Gallic Acid Content



Error bar:  $\pm$  Standard Error

## Hydrolyzed Gallic Acid

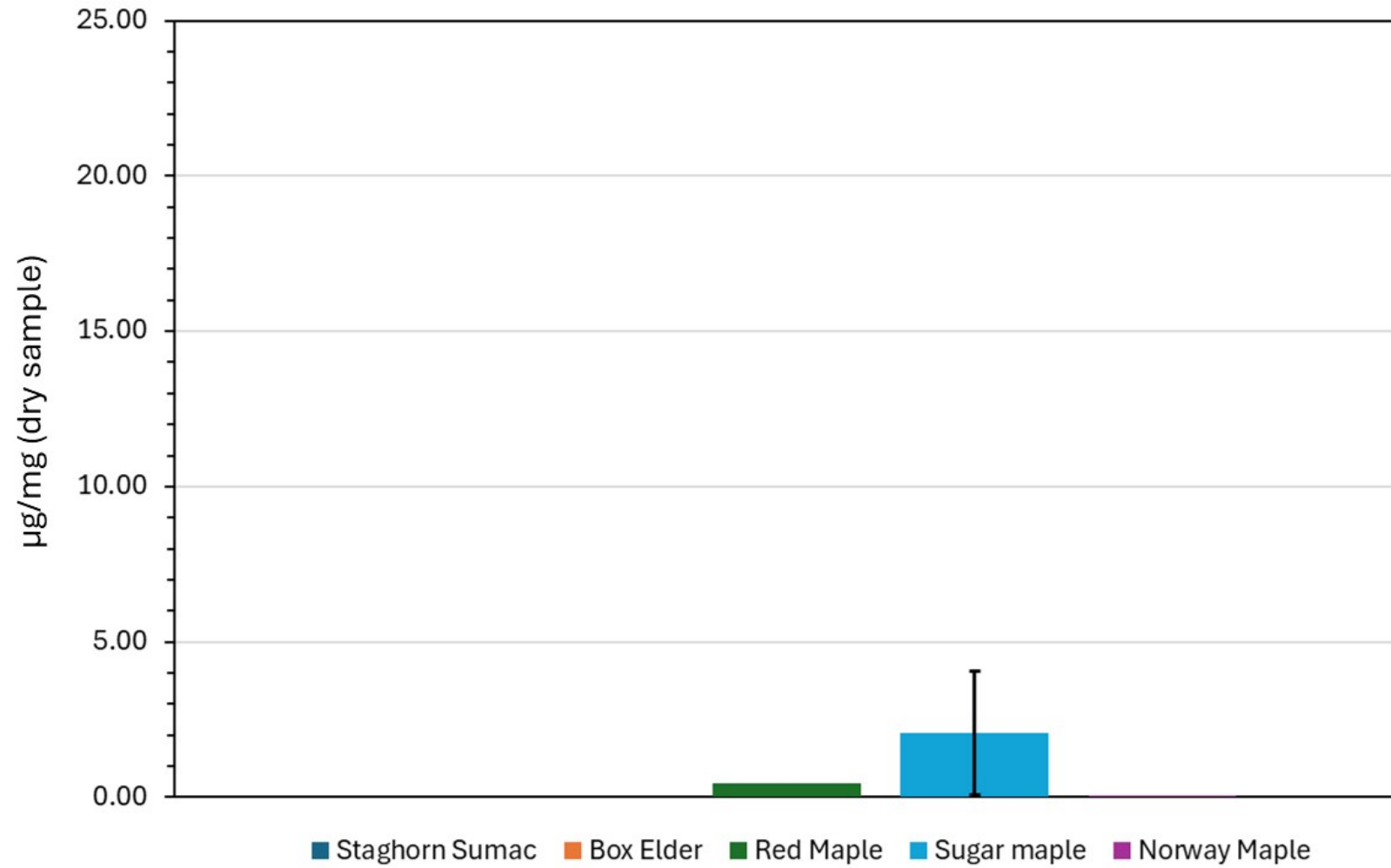


Zhentian Lei told me that Hydrolyzed = Total Gallic Acid. If so, these levels are inconsistent with Free Gallic Acid levels, which are much higher than these.

Error bar standard deviation

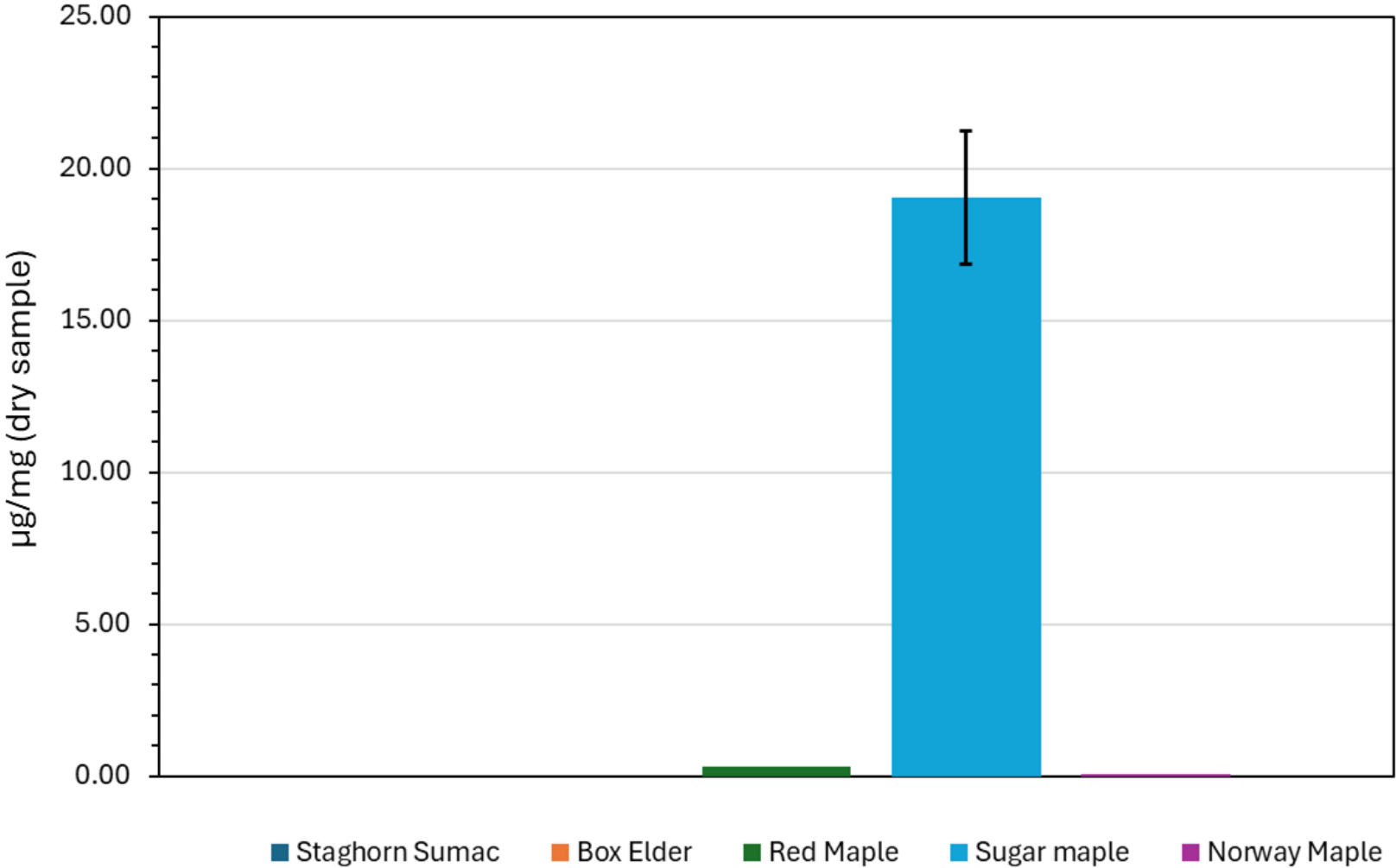
Sample	Date of harvest	Species	Site	Hydrolyzed gallic acid µg/mg
A1	06/24/2024	Staghorn Sumac	Belfast Rail Trail, Belfast	5.451252408
B1 <sup>a</sup>	06/24/2024	Staghorn Sumac	Old Belmont Rd., Lincolntown	52.13022035
D1	06/25/2024	Staghorn Sumac	Y Knot Farm, Belmont	24.98073218
A2	06/24/2024	Box Elder	Belfast Rail Trail, Belfast	5.475890325
E2	06/28/2024	Box Elder	Hunt Rd., Unity	5.013690818
F2	06/28/2024	Box Elder	MOFG Kitchen, Unity	33.75728155
A3	06/24/2024	Red Maple	Belfast Rail Trail, Belfast	26.21240556
C3	06/25/2024	Red Maple	3 Streams Farm, Belfast	21.39712531
D3	06/25/2024	Red Maple	Y Knot Farm, Belmont	6.074444482
A4	06/24/2024	Sugar maple	Belfast Rail Trail, Belfast	0.323529412
C4	06/25/2024	Sugar maple	3 Streams Farm, Belfast	4.256360078
D4	06/25/2024	Sugar maple	Y Knot Farm, Belmont	7.101310531
A5	06/24/2024	Norway Maple	Belfast Rail Trail, Belfast	26.88200047
C5	06/25/2024	Norway Maple	3 Streams Farm, Belfast	0.519607843
D5	06/25/2024	Norway Maple	Y Knot Farm, Belmont	0.557693037

### Free Ellagic acid



Error bar:  $\pm$  Standard Error

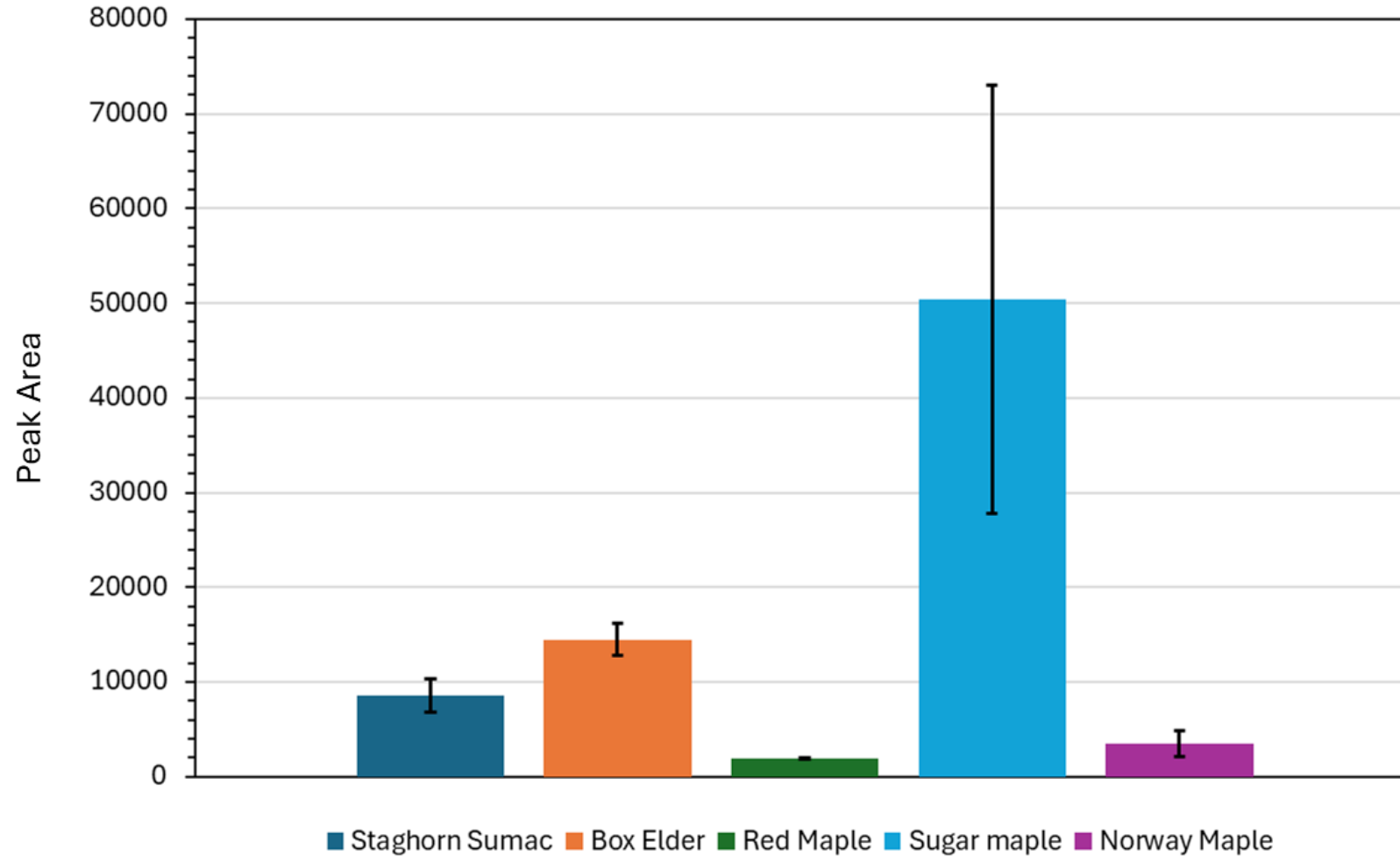
Hydrolyzed Ellagic acid



Error bar: ± Standard Error

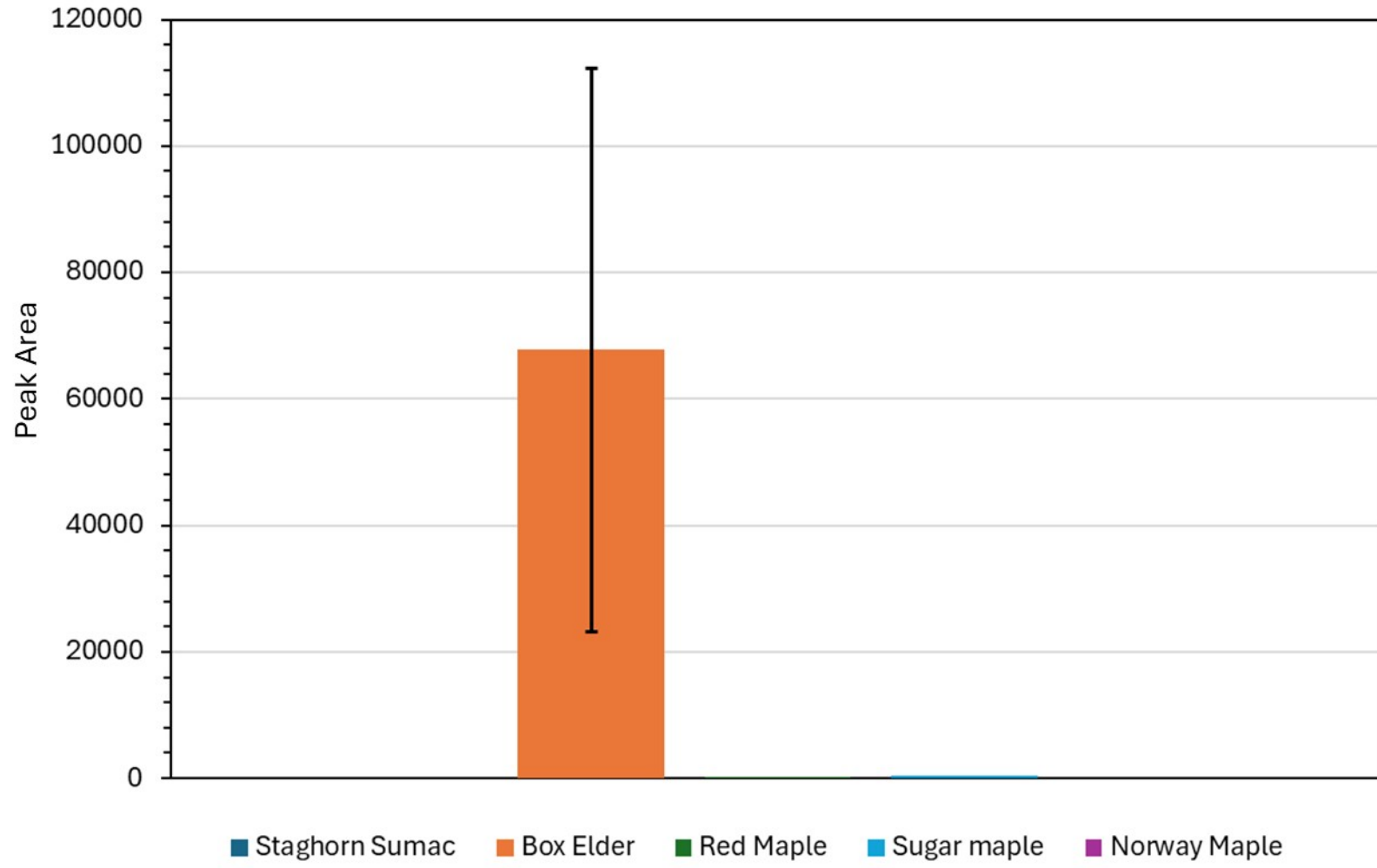


Average Peak Area of Hypoglycin A



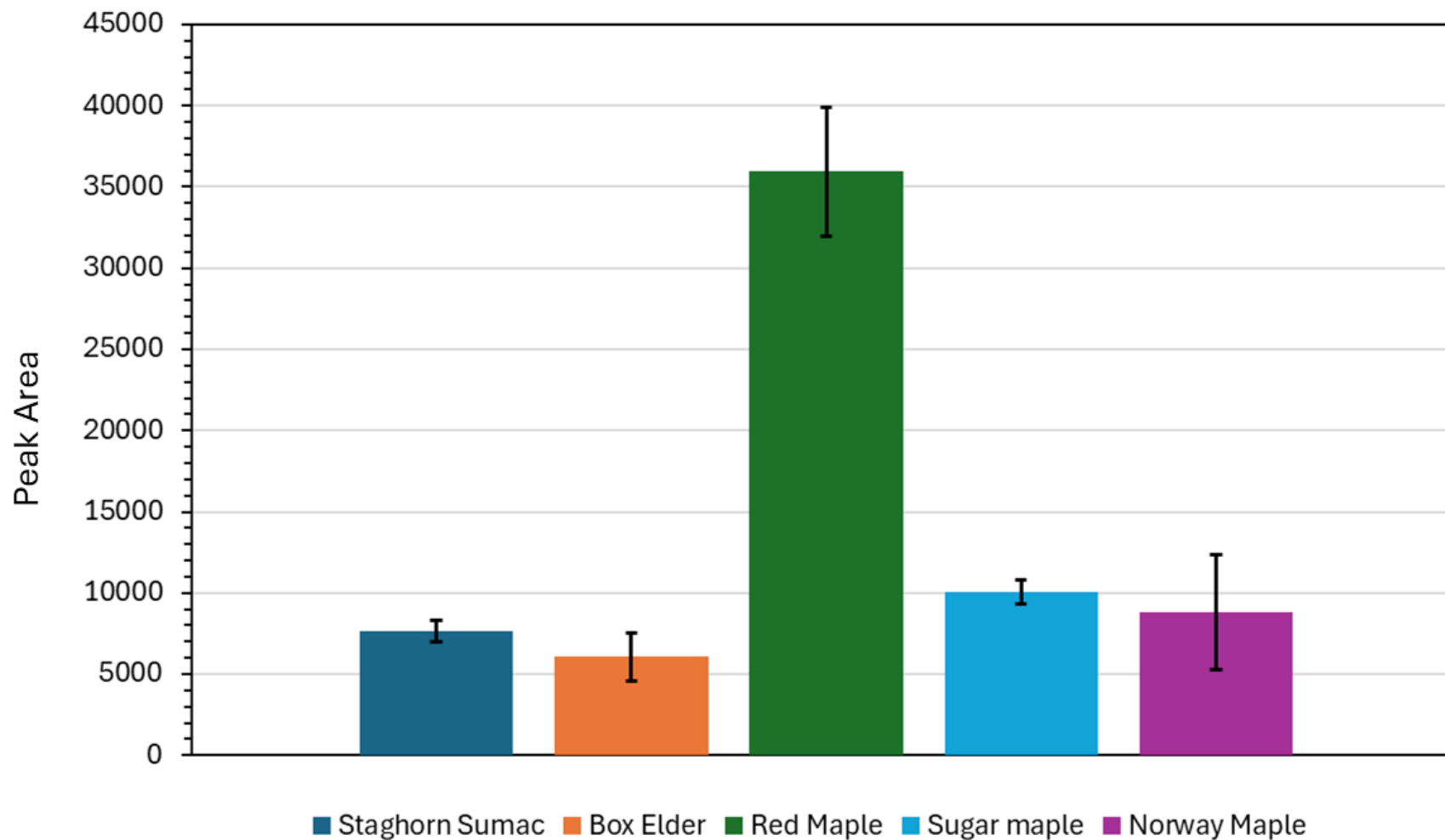
Error bar:  $\pm$  Standard Error

Average Peak Area of Hypoglycin B (HGB)



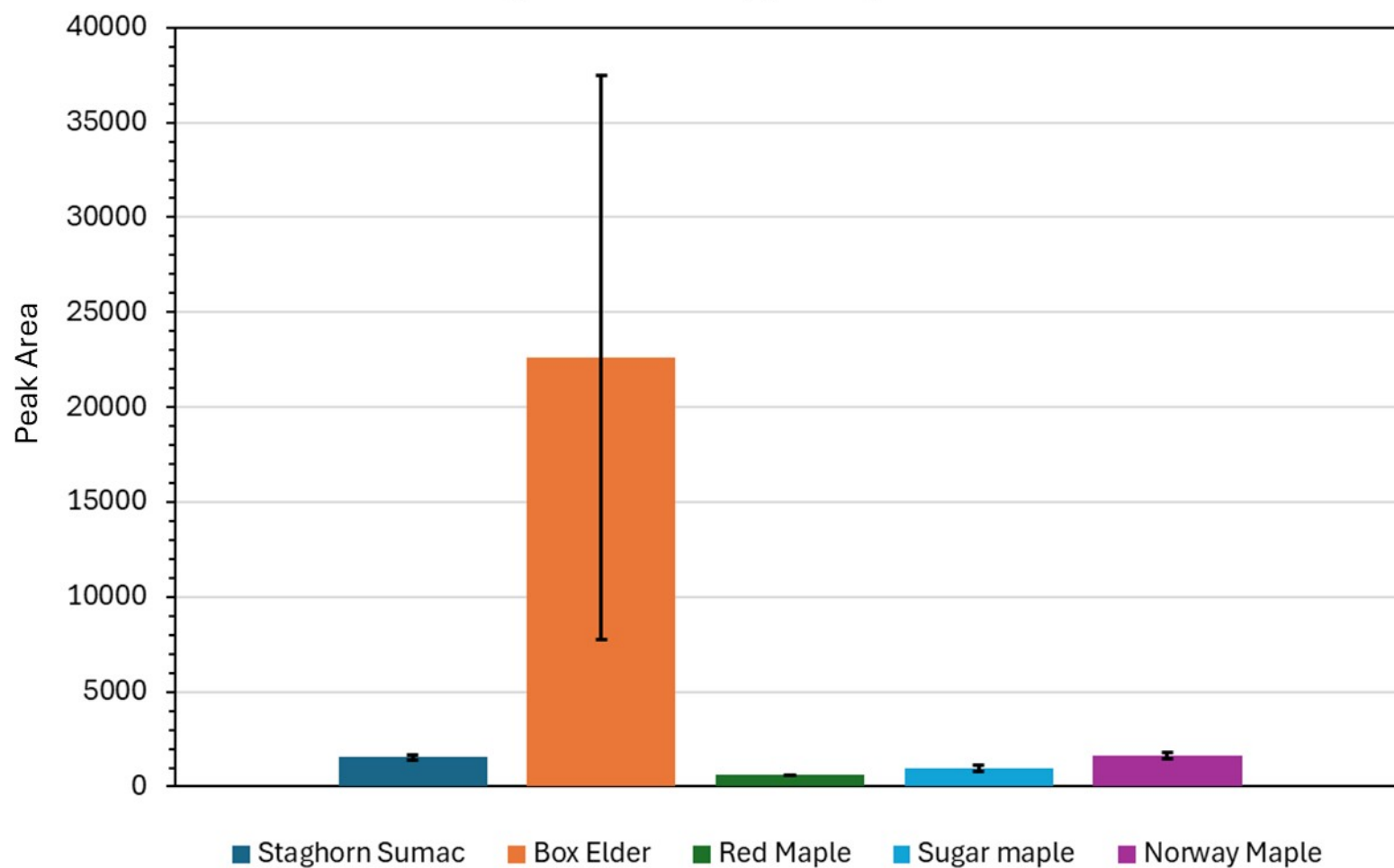
Error bar:  $\pm$  Standard Error

Average Peak Area of Methylene cyclopropylglycine (MCPPrG)



Error bar:  $\pm$  Standard Error

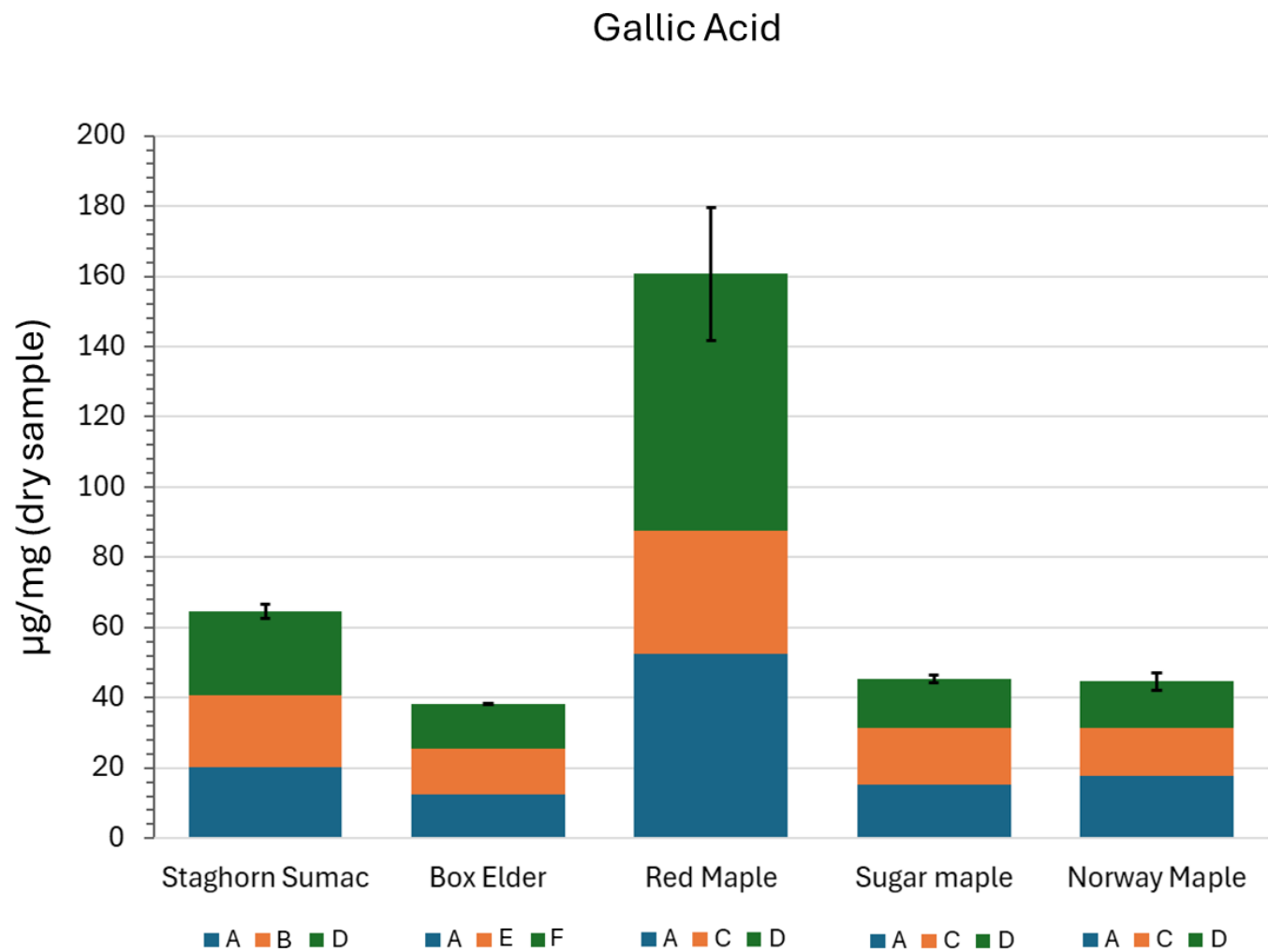
Average Peak Area of  $\gamma$ -glutamyl-MCPrGP



Error bar:  $\pm$  Standard Error

The concentration or  
abundance of the analyzed  
compounds in each sample

# Free?\* Gallic Acid Contents in Different Samples

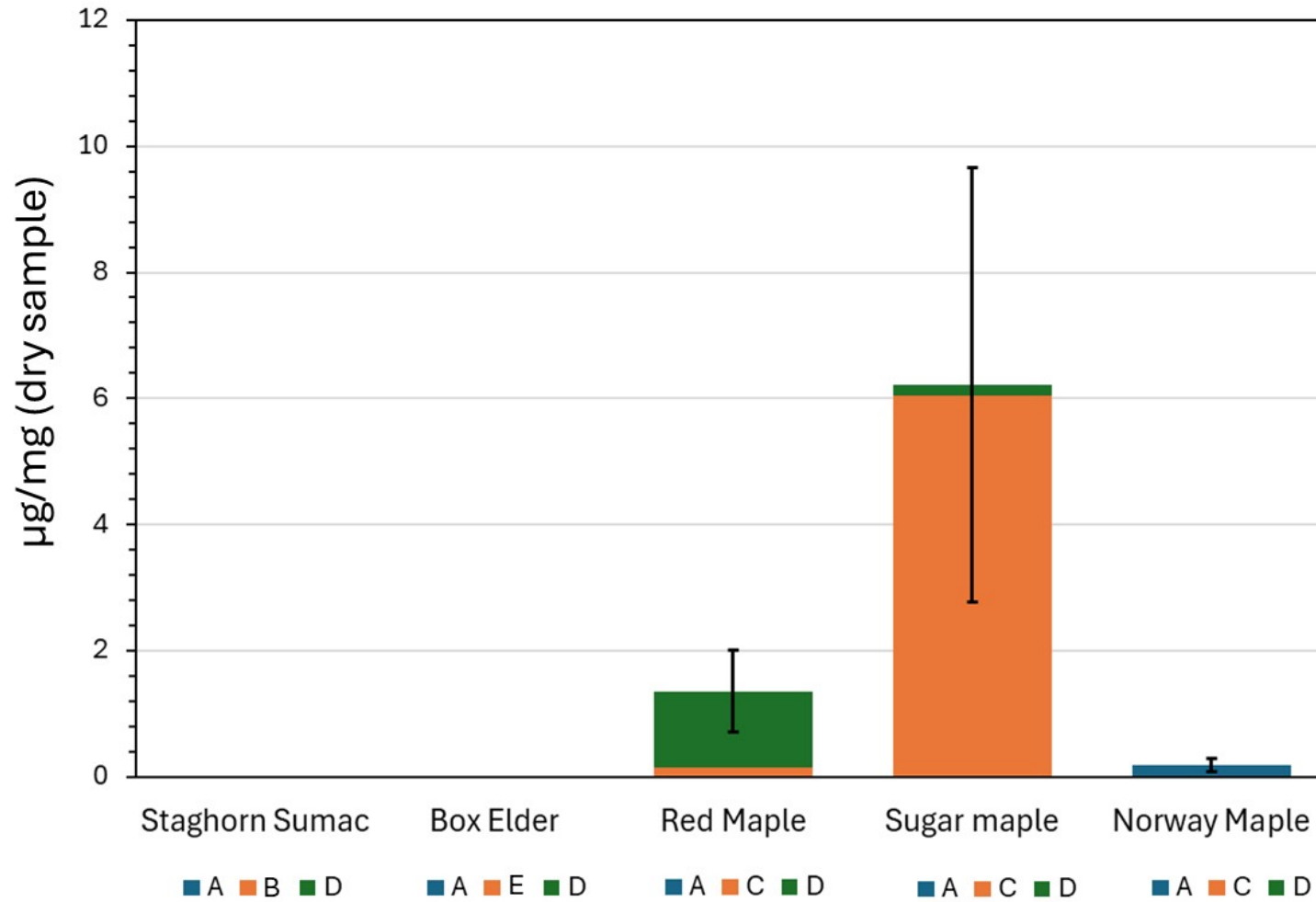


- A: Belfast Rail Trail, Belfast
- B: Old Belmont Rd., Lincolnville
- C: 3 Streams Farm, Belfast
- D: Y Knot Farm, Belmont
- E: Hunt Rd., Unity
- F: MOFG Kitchen, Unity

\* Zhentin forgot to include Hydrolyzed Gallic Acid in initial analyses and in this presentation. I have added his subsequent 2 slides of Hydrolyzed Gallic Acid. I added the word "Free" here, but not sure if that is correct. His Hydrolyzed or Total Gallic Acid levels are higher than the Free levels.

Error bar:  $\pm$ Standard Deviation

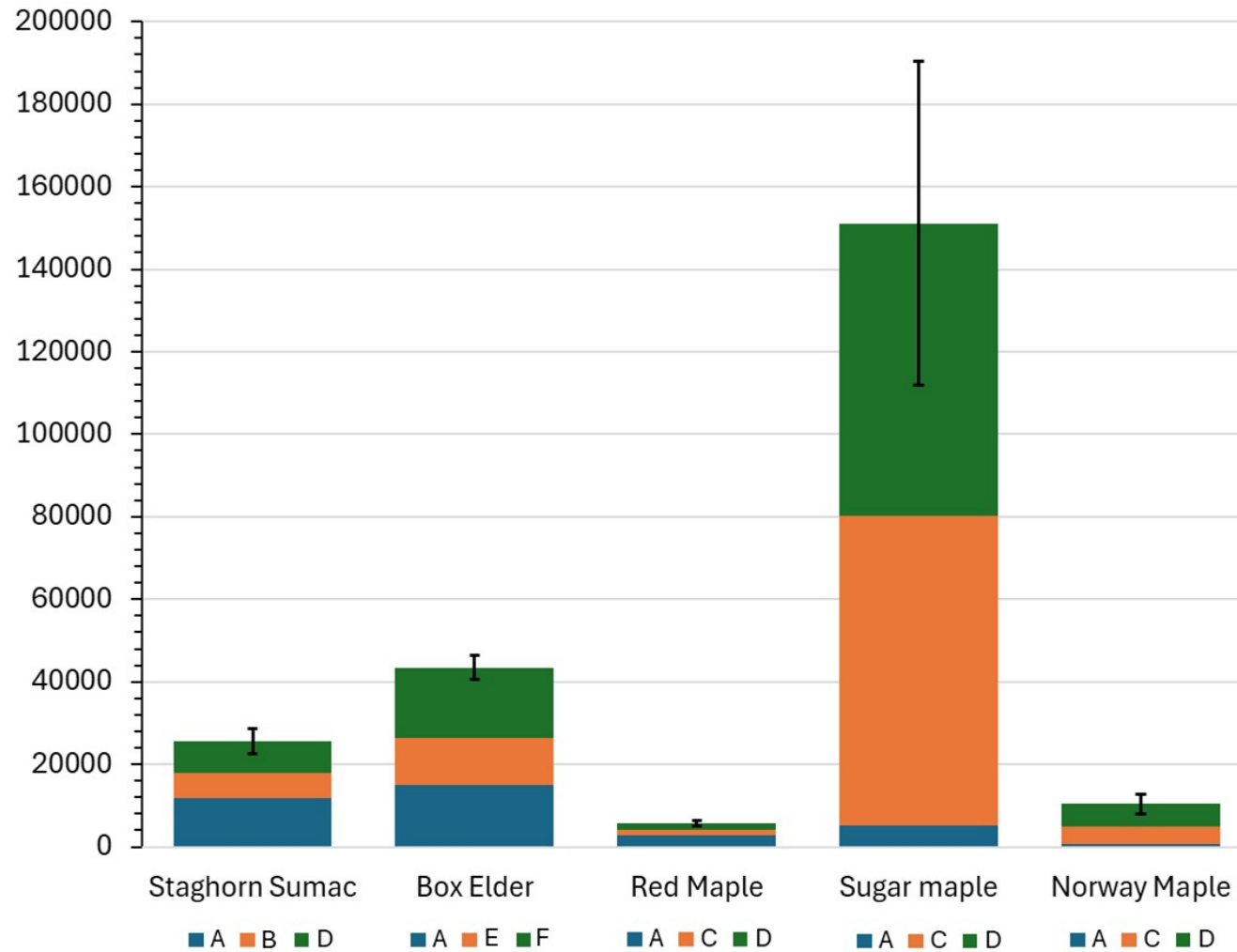
### Free Ellagic Acid



A: Belfast Rail Trail, Belfast  
B: Old Belmont Rd.,  
Lincolntown  
C: 3 Streams Farm, Belfast  
D: Y Knot Farm, Belmont  
E: Hunt Rd., Unity  
F: MOFG Kitchen, Unity

Error bar:  $\pm$ Standard Deviation

### Hypoglycin A (HGA) Peak Area

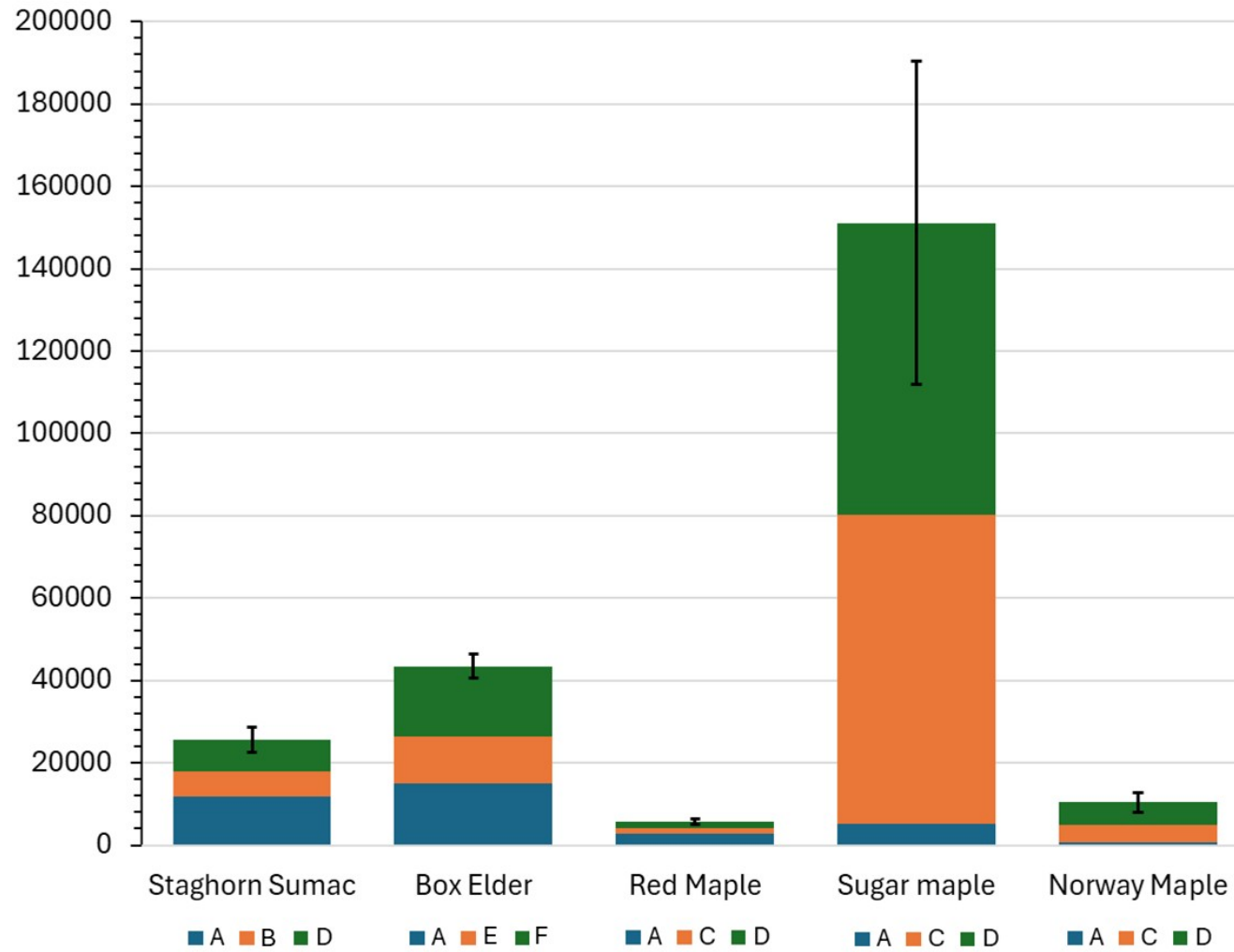


A: Belfast Rail Trail, Belfast  
B: Old Belmont Rd.,  
Lincolnvile  
C: 3 Streams Farm, Belfast  
D: Y Knot Farm, Belmont  
E: Hunt Rd., Unity  
F: MOFG Kitchen, Unity

Error bar:  $\pm$ Standard Deviation



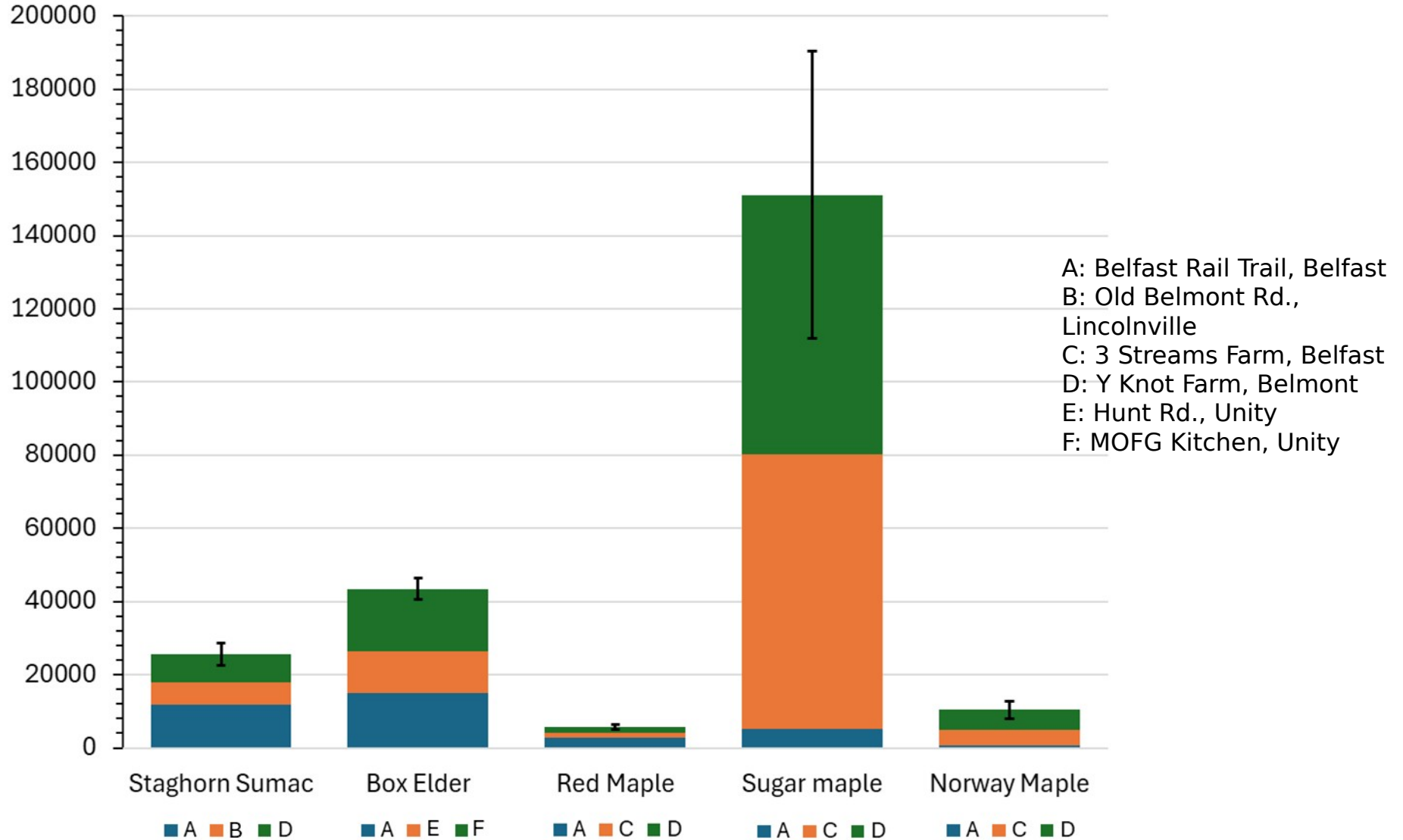
### Hypoglycin A (HGA) Peak Area



A: Belfast Rail Trail, Belfast  
B: Old Belmont Rd.,  
Lincolnvillie  
C: 3 Streams Farm, Belfast  
D: Y Knot Farm, Belmont  
E: Hunt Rd., Unity  
F: MOFG Kitchen, Unity

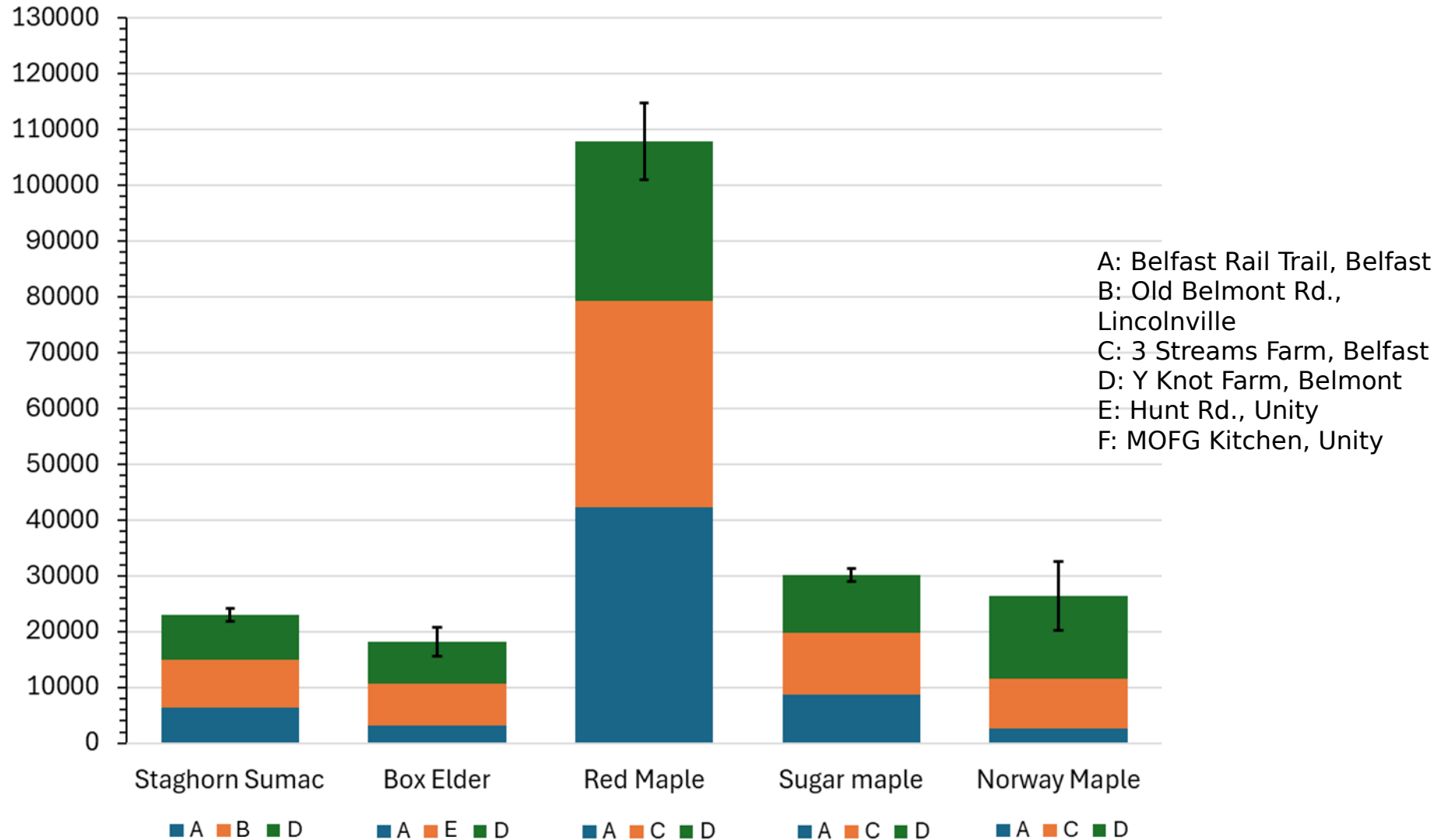
Error bar:  $\pm$ Standard Deviation

# Hypoglycin A (HGA) Peak Area



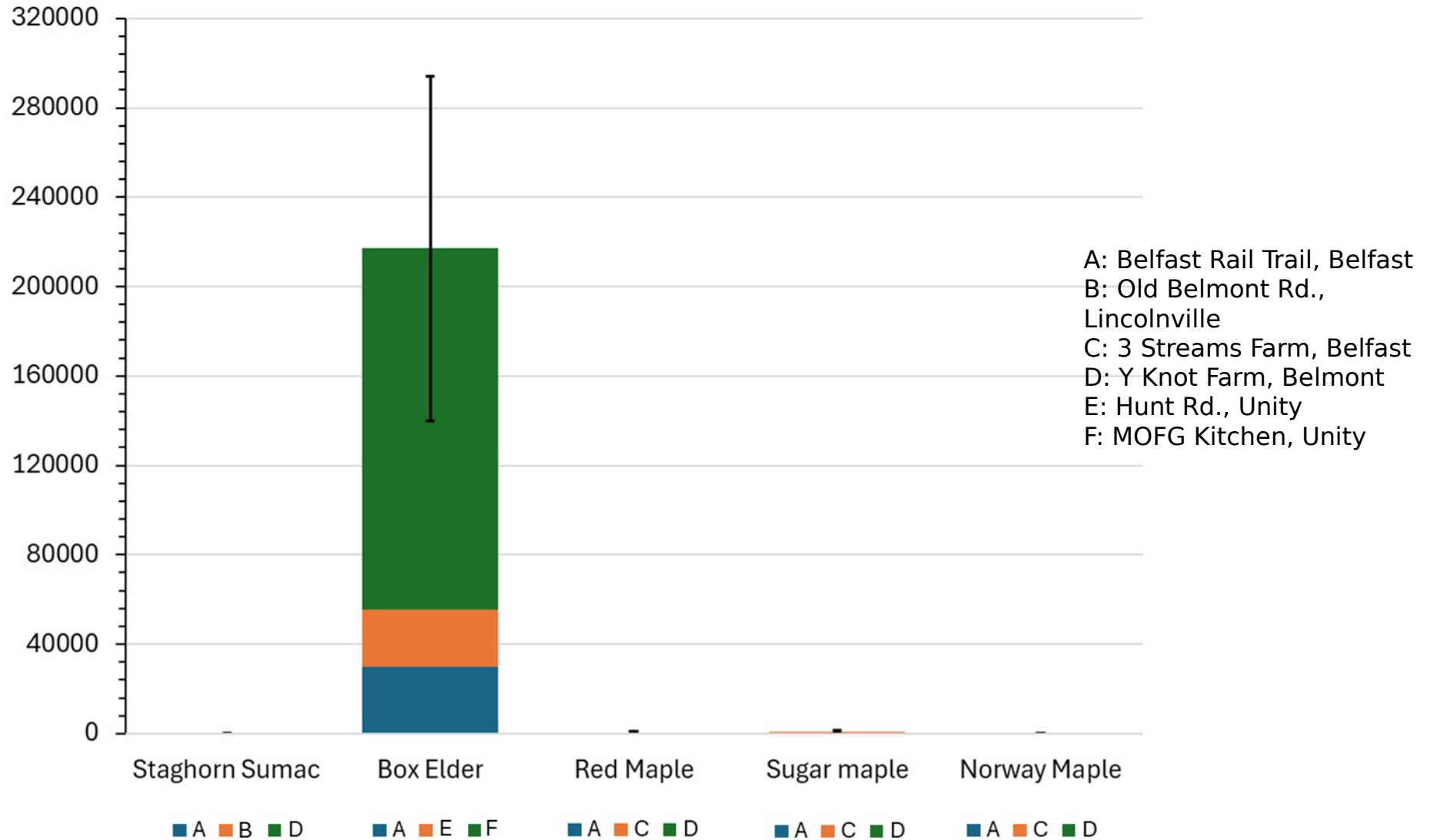
Error bar:  $\pm$ Standard Deviation

# Methylenecyclopropylglycine (MCPPrG) Peak Area



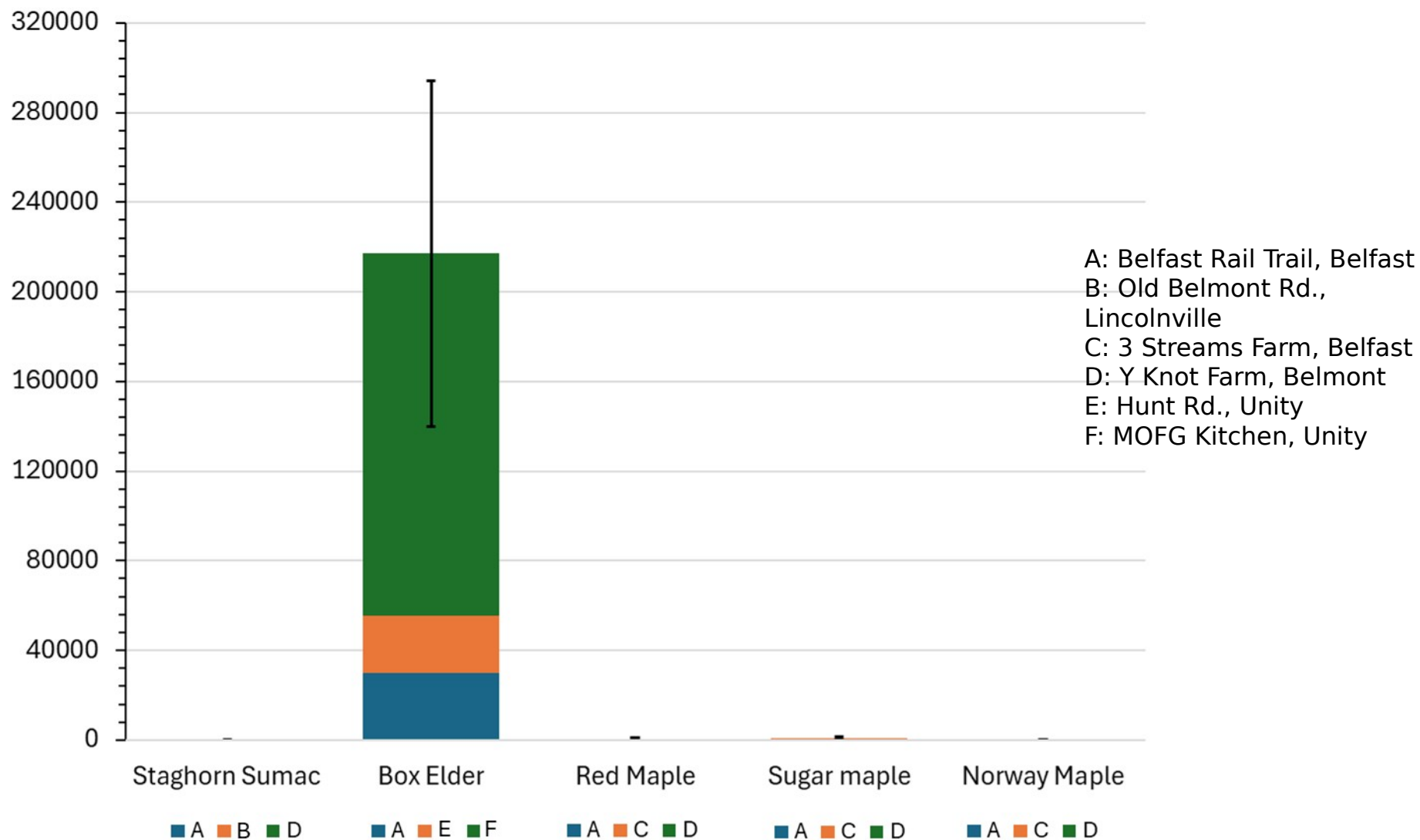
Error bar:  $\pm$  Standard Deviation

# Hypoglycin B (HGB) Peak Area



Error bar:  $\pm$  Standard Deviation

# γ-glutamyl-MCPrG Peak Area



Error bar:  $\pm$  Standard Deviation