

Public Abstract

First Name: Mitch

Middle Name: Curtiss

Last Name: Johnson

Adviser's First Name: C. Michael

Adviser's Last Name: Greenlief

Co-Adviser's First Name:

Co-Adviser's Last Name:

Graduation Term: FS 2016

Department: Chemistry

Degree: PhD

Title: LC-MS/MS Method Development for Quantification of Bioactive Compounds in Elderberry and Garlic Botanicals

Elderberry (*Sambucus nigra* spp.) juice contains a variety of polyphenols mostly anthocyanins. In order to understand the variation of polyphenol levels by genotype, various elderberry juice samples were analyzed for total phenolics (TP), total monomeric anthocyanins (TMA) and individual anthocyanin content (IAC). The Folin-Ciocalteu total phenolic method and pH differential method were used to measure the TP and TMA content, respectively. In addition, ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry was used to separate and detect individual anthocyanins from samples prepared by solid phase extraction. Multiple-reaction-monitoring was used to process data for the reduction of false positives, maximizing selectivity, and reliable quantification. The quantitative performance of the method was validated, and a detection limit of 0.3 ng/mL for cyanidin 3-O-glucoside was determined. This newly developed method may serve to characterize and profile various anthocyanins in elderberry juices for quality control, assessment of dietary intake, and anthocyanin-based biomedical studies.

The effects of frozen storage on the anthocyanin and polyphenol content of elderberry fruit juice are investigated. Juice from three genotypes of American elderberry (Adams II, Bob Gordon, and Wyldewood) was screened for total phenolic and total monomeric anthocyanin content with spectrophotometric methods. The individual anthocyanin content of the juice was tested by coupling solid phase extraction with ultra-performance liquid chromatography/tandem mass spectrometry. Juice samples were tested initially upon harvest, then again after 3, 6, and 9 months of frozen storage. The three different genotypes of juice had significantly different TP, TMA, and IAC profiles initially ($p < 0.05$). The TP, TMA, and IAC content of different genotypes were significantly affected ($p < 0.05$) by the frozen storage time, suggesting that both genotype and length of frozen storage time can affect the anthocyanin content of elderberry fruit juice.

Garlic is a prevalent plant botanical, and aged garlic extract (AGE) has been used as a nutritional supplement and implied to promote health benefits by exhibiting anti-oxidant and anti-inflammatory activity, as well as hypolipidemic and antiplatelet effects. It has recently been discovered that N^ω-(1-deoxy-D-fructos-1-yl)-L-arginine (FruArg) is a major contributor to the bioactivity of AGE and exerts significant ability in regulation of Nrf2-mediated antioxidant response. A very sensitive analytical method was developed and optimized for quantitation of FruArg in rat plasma and brain tissue samples. Phree™ phospholipid removal solution was used to separate samples spiked with FruArg from potentially interfering compounds present in biological fluids. Eluates were collected and analyzed using ultra-performance liquid chromatography-tandem mass spectrometry. A full method validation was conducted including analysis of the limit of quantitation, selectivity, linearity, range, recovery, matrix effect, inter-day precision and intra-day precision for both plasma and brain tissue. This method was applied to pharmacokinetic study where mice plasma and tissue samples from four regions of the brain were analyzed for FruArg concentration at 15, 30, 60, and 180 min after being injected intraperitoneally with FruArg. It was determined that FruArg is well absorbed into the bloodstream and detected in four sub-regions of the brain suggesting it crosses the blood-brain barrier (BBB).

Cyanogenic glycosides (CG) are present in a variety of plants and can rapidly breakdown to hydrogen cyanide, which is very toxic to human beings. The potential CG content of all parts of elderberry plant is not

well understood. In order to investigate the occurrence of CGs in elderberry ultra-performance liquid chromatography-tandem mass spectrometry methods were developed in order to quantitate several individual CGs at very low concentration levels, which is a large improvement in the current methodology. Upon harvest, the seeds, skin, juice, pulp and stems of elderberry will be analyzed for the presence of CGs.