The effect of Anthocyanin-rich purple corn extract on the healthspan of *Caenorhabditis elegans*

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Introduction

If aging is a result of the degradation of the proteostasis network (PN), then treatments that support proteostasis are expected to increase healthspan. Anthocyanins have been credited with the capacity to modulate cognitive and motor function, enhance memory and having a role in preventing age-related declines in neural function (Chen et al., 2013). We are studying the effect of standardized purple corn extract on the CL4176 transgenic strain of *C.elegans,* in order to observe an increase in healthspan. *C.elegans* are used as a model organism due to their short life span.

Figure:1 The IGF-1 signaling cascade (Chen et al., 2013).

The insulin/insulin-like growth factor (IGF-1) pathway regulates the signaling cascade which influences aging in animals (Figure 1). The cascade is effected by anthocyanins leading to a translocation of DAF-16 into the nucleus, where it stimulates the expression of stress resistance and longevity-related genes (Chen et al., 2013).

Purple corn (*Zea mays Indurata*) contains 1,642mg anthocyanin content per 100g. This is one of the highest contents among plants (Kano et al., 2005). Anthocyanins are made up of polyphenols which have been seen to have antioxidant health benefits in humans (Cuevas Montilla et al., 2011). HPLC was used in order to identify and quantify key compounds within the purple corn.

Methods

Setting up the populations

CL4176 worms were chunked onto Nematode growth media (NGM Plates) seeded with *E.coli* strain OP50 as a food source.

Experimental plates

NGM in solution, NaOH and H_2O were mixed in individual test tubes in order to control pH and quantity of solution. Solution was sterilized via autoclaving.

Purple corn was added to NGM, 0.1%, 0.2%, 0.4%, caffeine (positive control) and Control (negative control) were made.

Counting

Synchronized worms were left 48 hours at 16^{0}C in order to grow into L3 worms, before upshifting to 25^{0}C for 20 hours. Worms were counted every 2 hours from then onwards. The CL4176 transgenic strain causes an overexpression of the β-amyloid protein, leading to protein, aggregation, paralysis and eventual death of the nematodes.

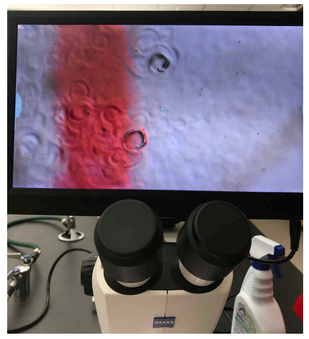

Figure 2: Shows *C.elegans* on a 0.4% anthocyanin concentration trial plate at hour 46

HPLC

Buffer containing methanol: acetic acid: water (50:8:42) was used to dissolve the maltodextrin. Solution was vortexed and ultrasonicated.

HPLC system was 1220 infinity II column (250mm x 4.6mm i.d. 5µl) flow rate 0.6ml/min. Solvent A (Formic acid/ water) Solvent B (Formic acid/ Acetonitrile).

Phases: 0min: 5% B
3min: 20% B
30min: 95% B
35min: 95% B
45min: 5% B
Run time: 50 minutes

Results

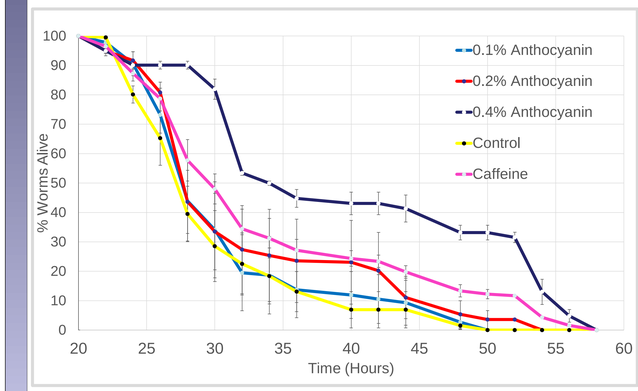

Figure 3: Lifetime assay: Effects of different anthocyanin concentrations (0.1-0.4%), untreated control plates, and 6.27mM Caffeine on % of Worms alive.

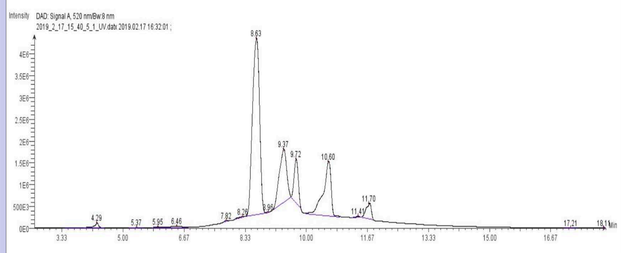

Figure 4: HPLC chromatogram of the anthocyanin-rich purple corn at 520 nm.

Table 1:Polyphenol content of purple corn extract.

Compound	rt	m+	Fragment
(epi)catechincyanidin-3,5-diglucoside	6.71	899	737,537,449
cyanidin-3-glucoside	8.86	449	
pelargonidin-3-glucoside	9.26	433	535,463,449
peonidin-3-glucoside	9.37	463	535,449
cyanidin-3-(6"-malonylglucoside)	9.53	535	463,449
pelargonidin-3-(6"-malonylglucoside)	9.91	519	549,535,519
peonidin-3-(6"-malonylglucoside)	10.01	549	535
cyanidin-3-(dimalonylglucoside)	10.08	621	535,519
pelargonidin-3-(dimalonylglucoside)	10.64	605	549
peonidin-3-(dimalonylglucoside)	10.71	635	549,463

Conclusions

- From our data there was a significant increase (P=<0.01) in the healthspan of *C. elegans.*
- We see a 12% increase in lifespan with the addition of 0.4% purple corn. We also see a large healthspan increase in the *C.elegans* up to 52 hours, with the addition of 0.4% anthocyanin, thus showing that the anthocyanin-rich purple corn extract increases the healthspan of *C.elegans.*

Future work

- Run elderberry lifetime assay alongside purple corn to see the differences in healthspan among two anthocyanin containing plants.
- HPLC & mass spectrometry of elderberry, for comparison of the different anthocyanin compounds. Allowing for a comparison of specific active compounds between the two extracts to explain differences in healthspan extension.
- Research will be conducted to examine the effects of maltodextrin on the healthspan of *C.elegans.*

References

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