Producing Nutraceutical Carotenoids in Soybean Seeds

Danforth Plant Science Center

Carotenoids in Nature

600 different carotenoids

$ 766 million global market, most are chemically synthesized

Colorant in food and feed

Anti-oxidant activity -- nutraceutical

Lipid soluble

Carotenoids in Foods

	Neoxanthins and violaxanthins	*Lutein and zeaxanthin*	*Lutein*	*Zeaxanthin*	*Cryptoxanthins*	*Lycopenes*	*α carotene*	*β carotene*
Egg yolk	8	89	54	35	4	0	0	0
Maize (corn)	9	86	60	25	5	0	0	0
Kiwi	38	54	54	0	0	0	0	8
Red seedless grapes	23	53	43	10	4	5	3	16
Zucchini squash	19	52	47	5	24	0	0	5
Pumpkin	30	49	49	0	0	0	0	21
Spinach	14	47	47	0	19	4	0	16
Orange pepper	4	45	8	37	22	0	8	21
Yellow squash	19	44	44	0	0	0	28	9
Cucumber	16	42	38	4	38	0	0	4
Pea	33	41	41	0	21	0	0	5
Green pepper	29	39	36	3	20	0	0	12
Red grape	27	37	33	4	29	0	1	6
Butternut squash	24	37	37	0	34	0	5	0
Orange juice	28	35	15	20	25	0	3	8
Honeydew	18	35	17	18	0	0	0	48
Celery (stalks, leaves)	12	34	32	2	40	1	13	0
Green grapes	10	31	25	7	52	0	0	7
Brussels sprouts	20	29	27	2	39	0	0	11
Scallions	32	29	27	3	35	4	0	0
Green beans	27	25	22	3	42	0	1	5
Orange	36	22	7	15	12	11	8	11
Broccoli	3	22	22	0	49	0	0	27
Apple (red delicious)	22	20	19	1	23	13	5	17
Mango	52	18	2	16	4	6	0	20
Green lettuce	33	15	15	0	36	0	16	0
Tomato juice	0	13	11	2	2	57	12	16
Peach	20	13	5	8	8	0	10	50
Yellow pepper	86	12	12	0	1	0	1	0
Nectarine	18	11	6	6	23	0	0	48
Red pepper	56	7	7	0	2	8	24	3
Tomato (fruit)	0	6	6	0	0	82	0	12
Carrots	0	2	2	0	0	0	43	55
Cantaloupe	9	1	1	0	0	3	0	87
Dried apricots	2	1	1	0	9	0	0	87
Green kidney beans	72	0	0	0	28	0	0	0

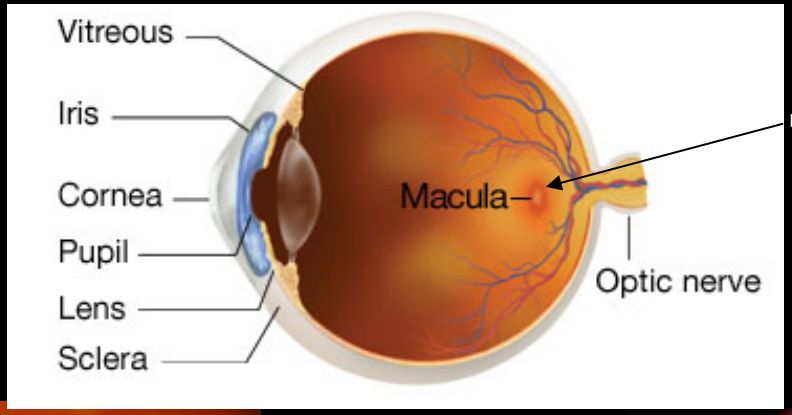
Values in mole%

Br J Opthlalmol 1998 82: 907-910

Potential Carotenoids in Soybean Seeds

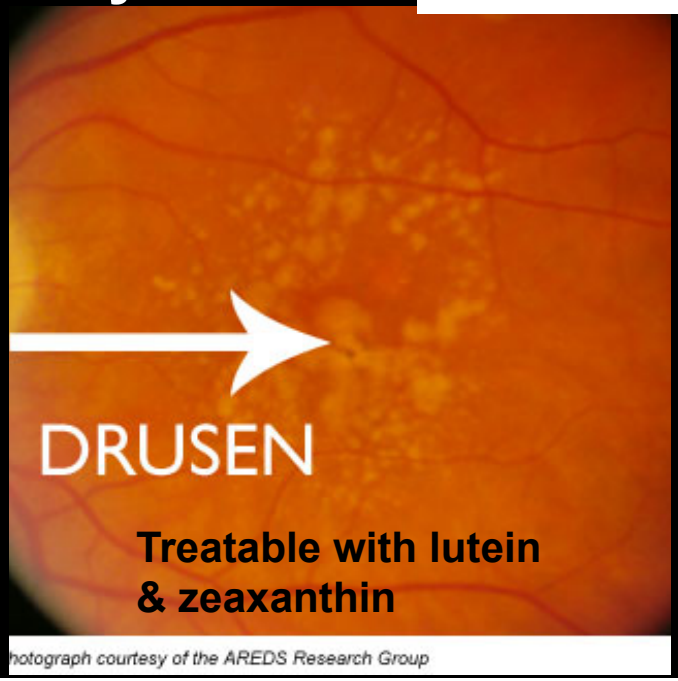
Age-related Macular Degeneration AMD

Leading cause of irreversible vision loss of Americans > 55 yrs
Currently affects 10 million Americans



lutein & zeaxanthin

Dry AMD

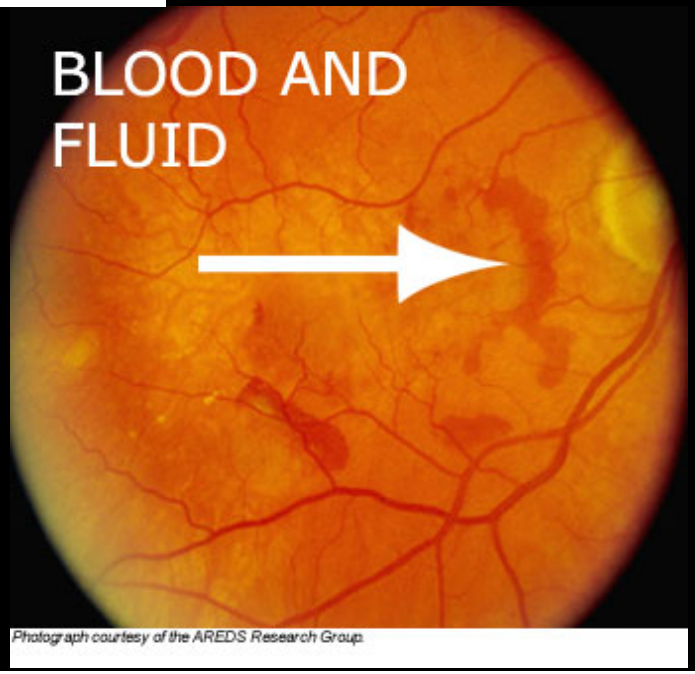


Treatable with lutein & zeaxanthin

hotograph courtesy of the AREDS Research Group

untreated

Wet AMD

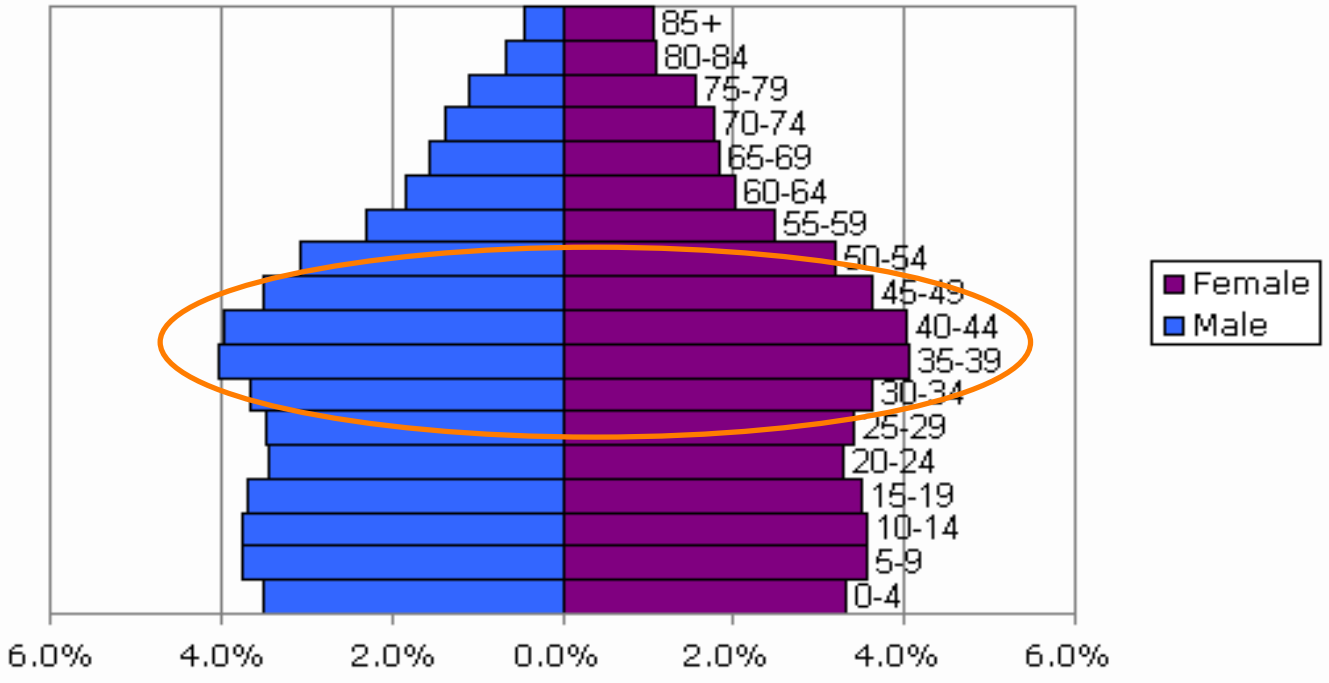


Photograph courtesy of the AREDS Research Group

Age Distribution of American Population ~2000

	Over 65	
1990	12 million	} Aging baby-boomers & increased life expectancy
2030	71 million	

6-10 mg daily intake of zeaxanthin in Americans would have $2.5 billion net savings to Medicare system over next 5 yrs (Lewin Study, 2006)



Potential Carotenoids in Soybean Seeds

Astaxanthin

Flamingo industry??
Inexplicably popular lawn ornaments in Florida

Synthetic astaxanthin sells for $2,000/kg;
Natural astaxanthin sells for $7,000/kg

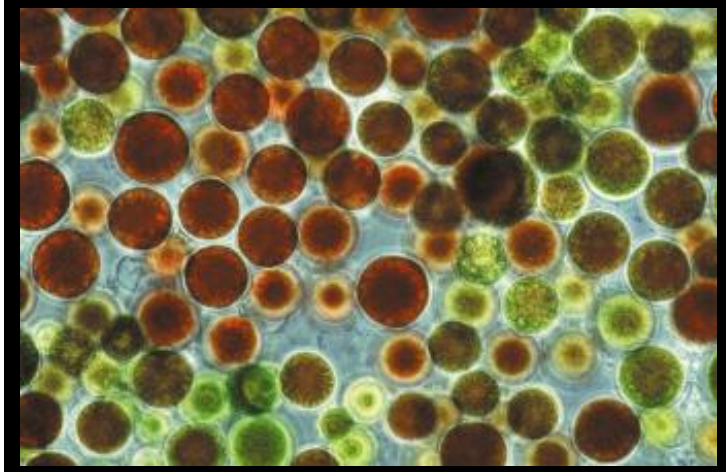


Photo from Fraunhofer IGB

Salmon production in the US in 2001 was estimated to be 46 million pounds and valued at $72 million
(Source: National Marine Fisheries Service)

Carotenoid supplements can be 15 – 25% of total feed costs

Carotenoid Biochemistry

Acetyl CoA

Many steps

Endogenous to soybean seeds

tocopherols

gibberellins

chlorophyll

Geranyl geranyl pyrophosphate (GGPP)

crtB transgene

Phytoene synthase

Phytoene

Phytoene desaturase

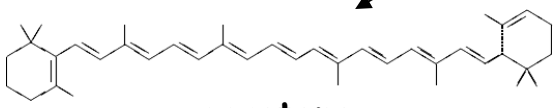
ζ-carotene

ζ-carotene desaturase

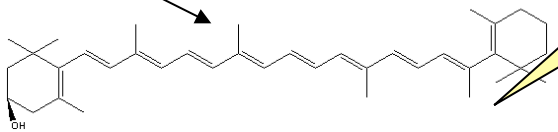
lycopene

lycopene β and ε cyclase

lycopene β cyclase



α-carotene



β-carotene

β-carotene
Pro-vitamin A
Replacing vitamin A in diary products

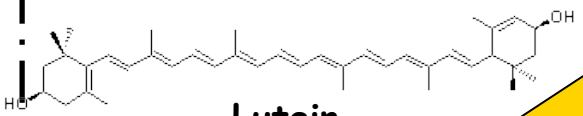
$ 242 million/yr

Canthaxanthin

Colorant in chickens (skin and yolk)

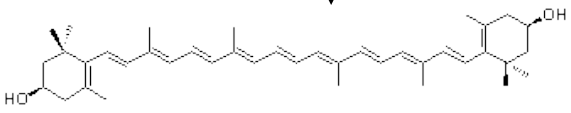
$ 100 million/yr

α-carotene hydroxylase



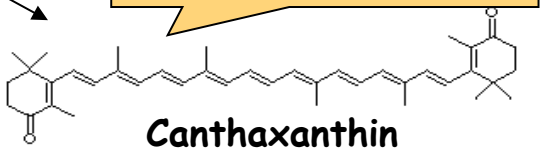
Lutein

β-carotene hydroxylase



Zeaxanthin

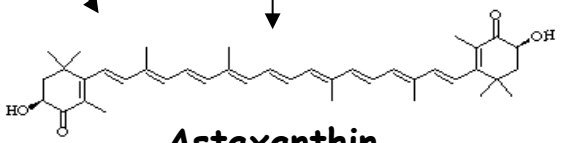
β-C 4 oxygenase



Canthaxanthin

β-carotene ketolase

β-C 3 hydroxygenase



Astaxanthin

Lutein and Zeaxanthin

Age-related macular degeneration
Nutritional supplements
$139 million/yr

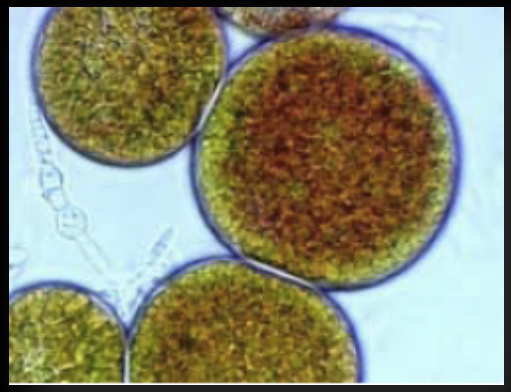
Astaxanthin
Salmon, shimp, lobster
~100X better antioxidant then vitaminE
$ 234 million/yr

Source of Carotenoid Genes

Have PCR, will travel

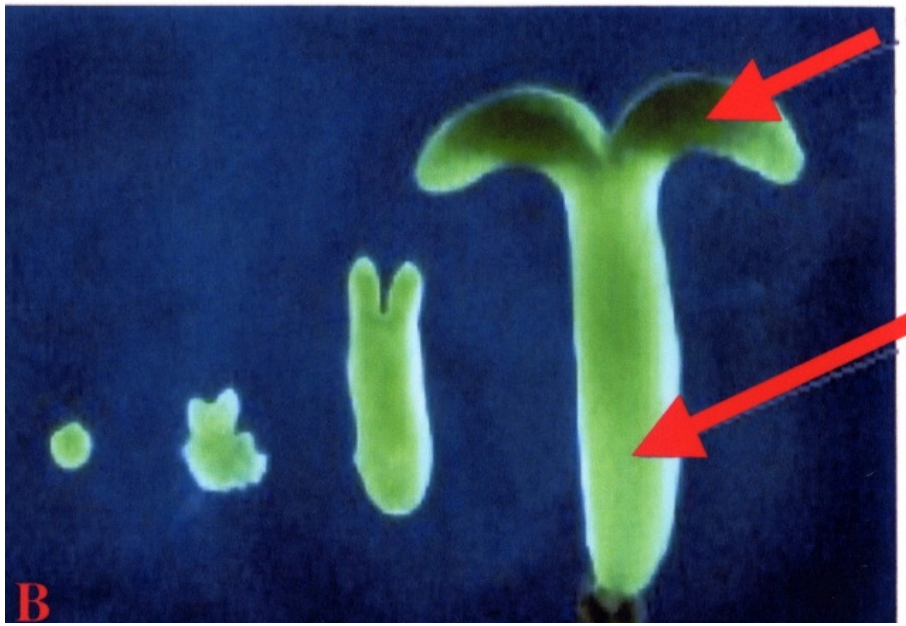
Adonis aestivalis

Xanthophyllomyces dendrorhous

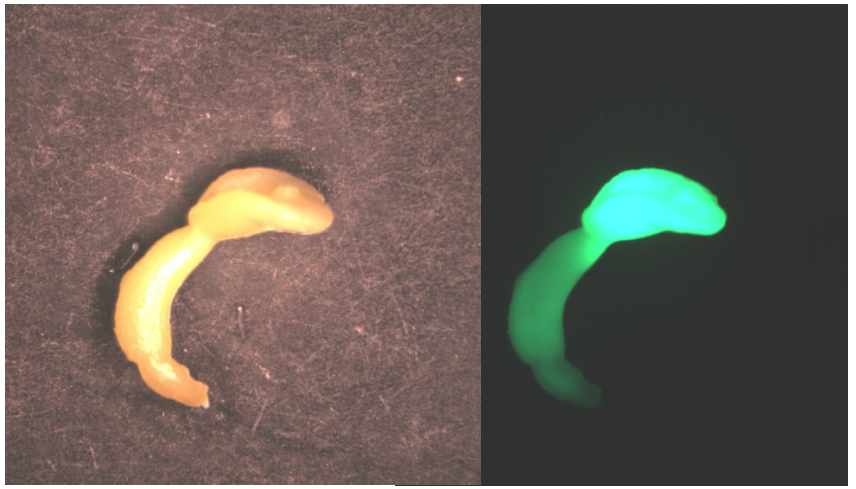


Haematococcus pluvialis

Somatic embryo system allows early phenotype detection for seed specific traits

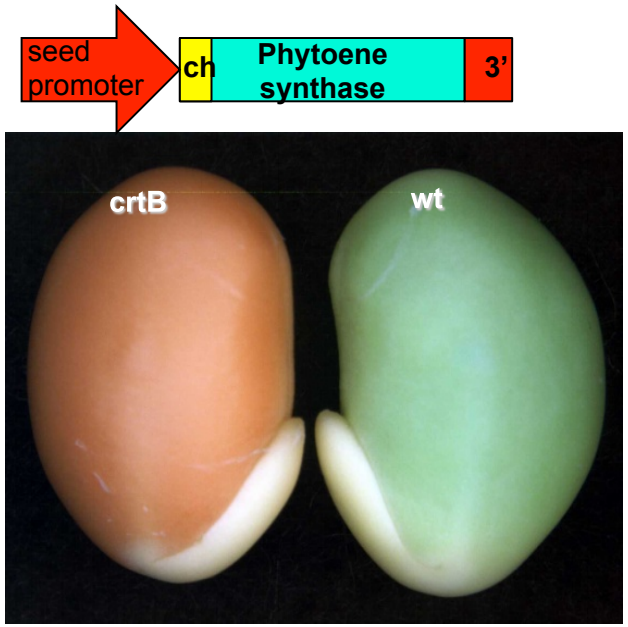


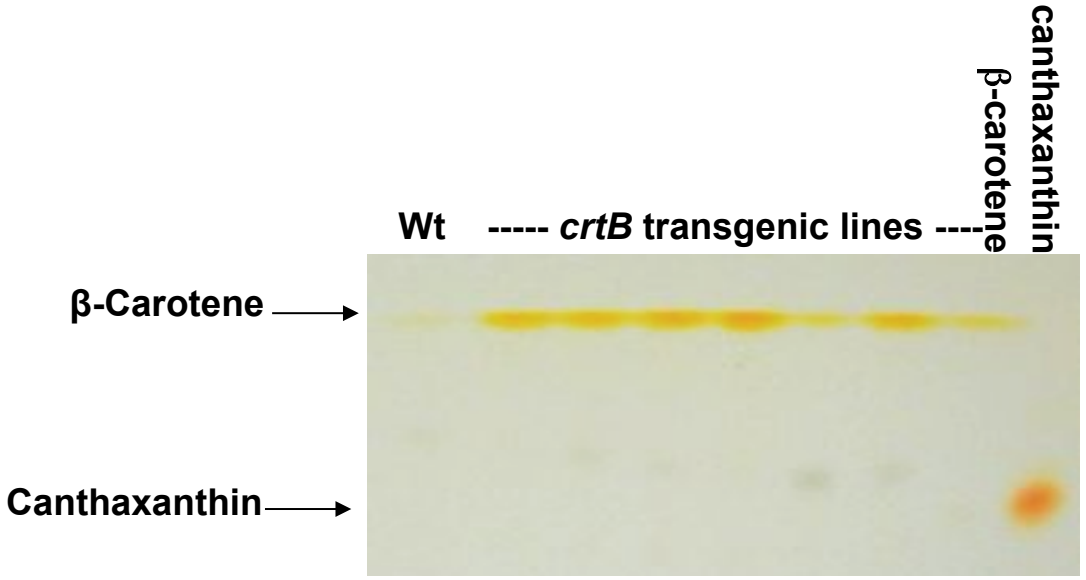
Example 1: GFP



Example 2: carotenoid

Carotenoids in Soybean Seeds



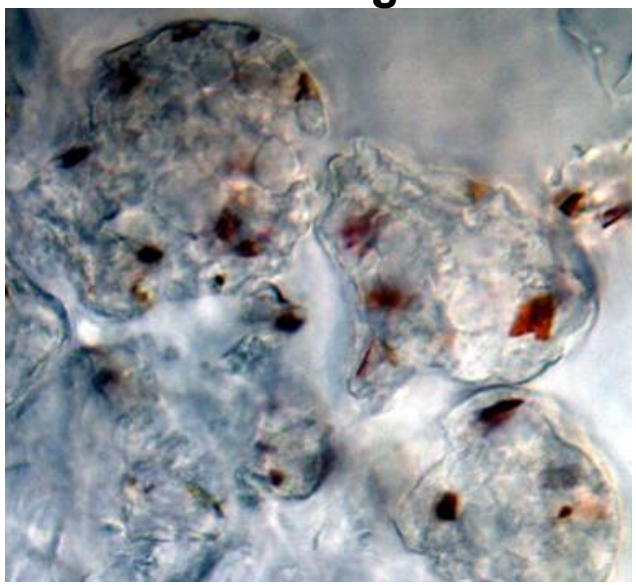


Over-expression of crtB gene in soybean seeds resulted in an enhanced level of β-carotene production.

This indicates phytoene synthase step is largely the rate limiting step in the carotenoid pathway in soybean seeds.

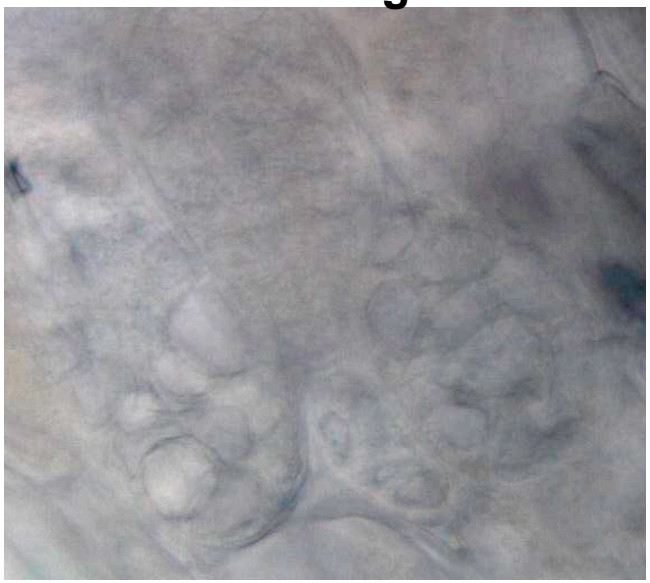
β-carotene accumulation as a result of metabolic engineering of the chloroplast isoprenoid pathway

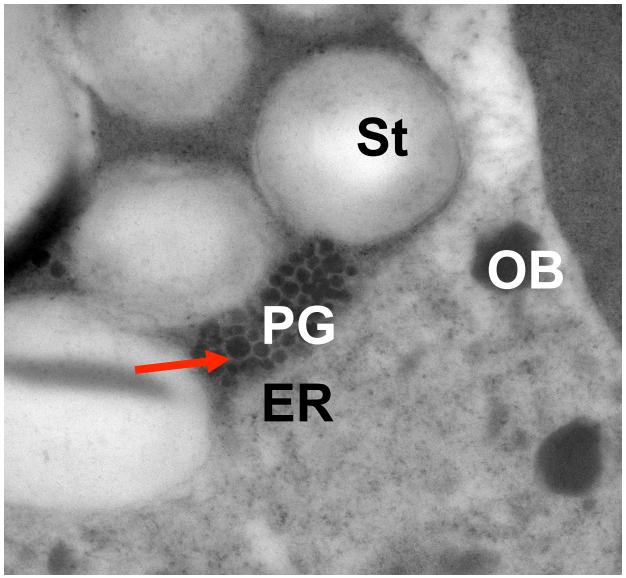
Crt B Transgenic



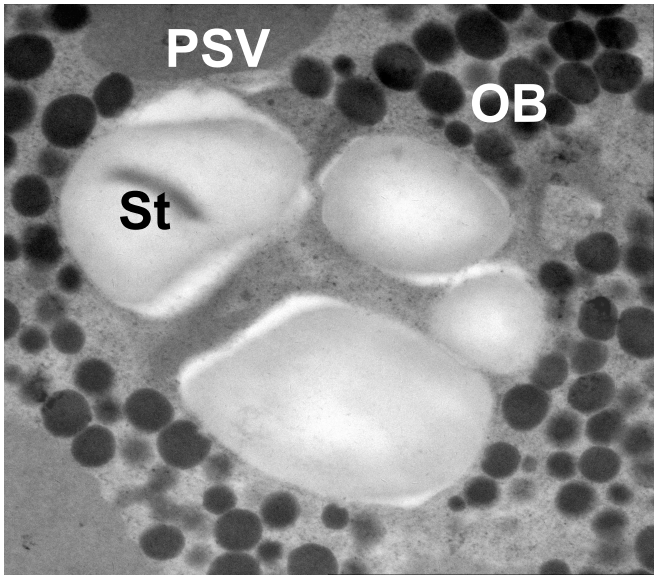
light microscopy

Non Transgenic

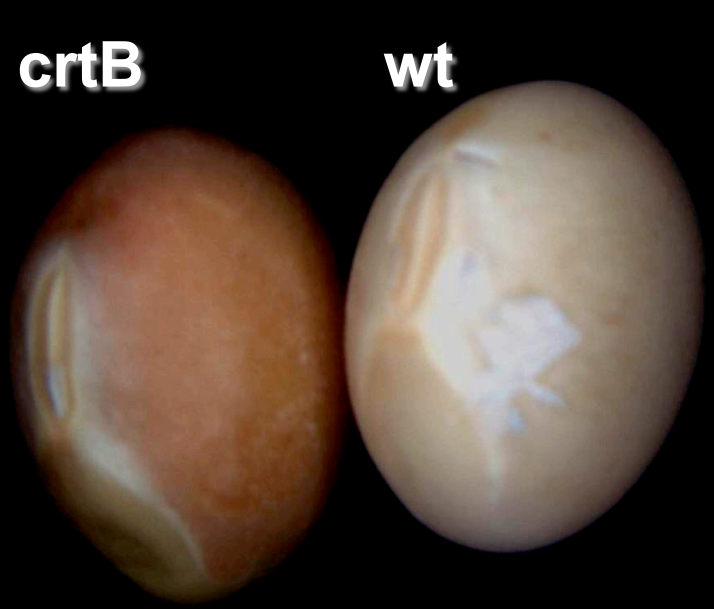


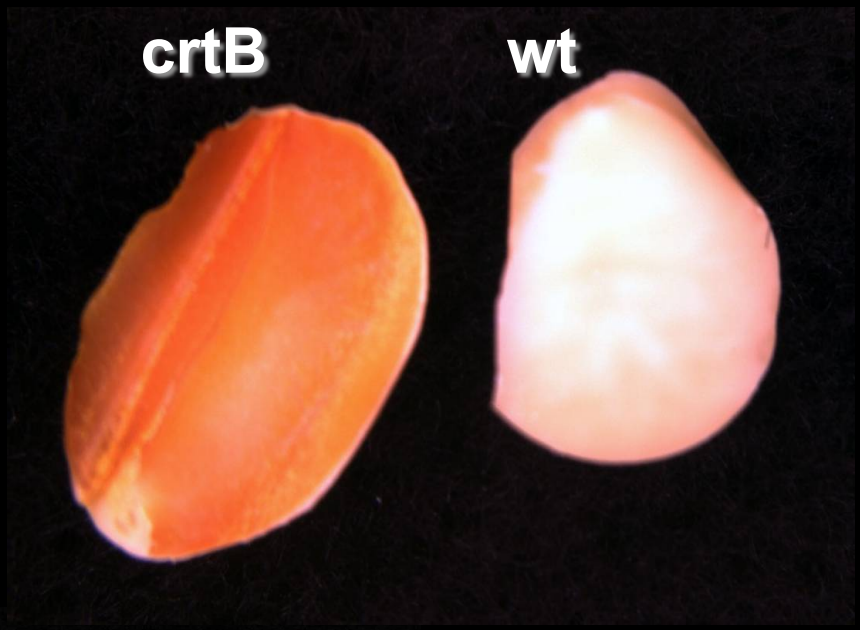


electron microscopy



Carotenoids in Soybean Seeds



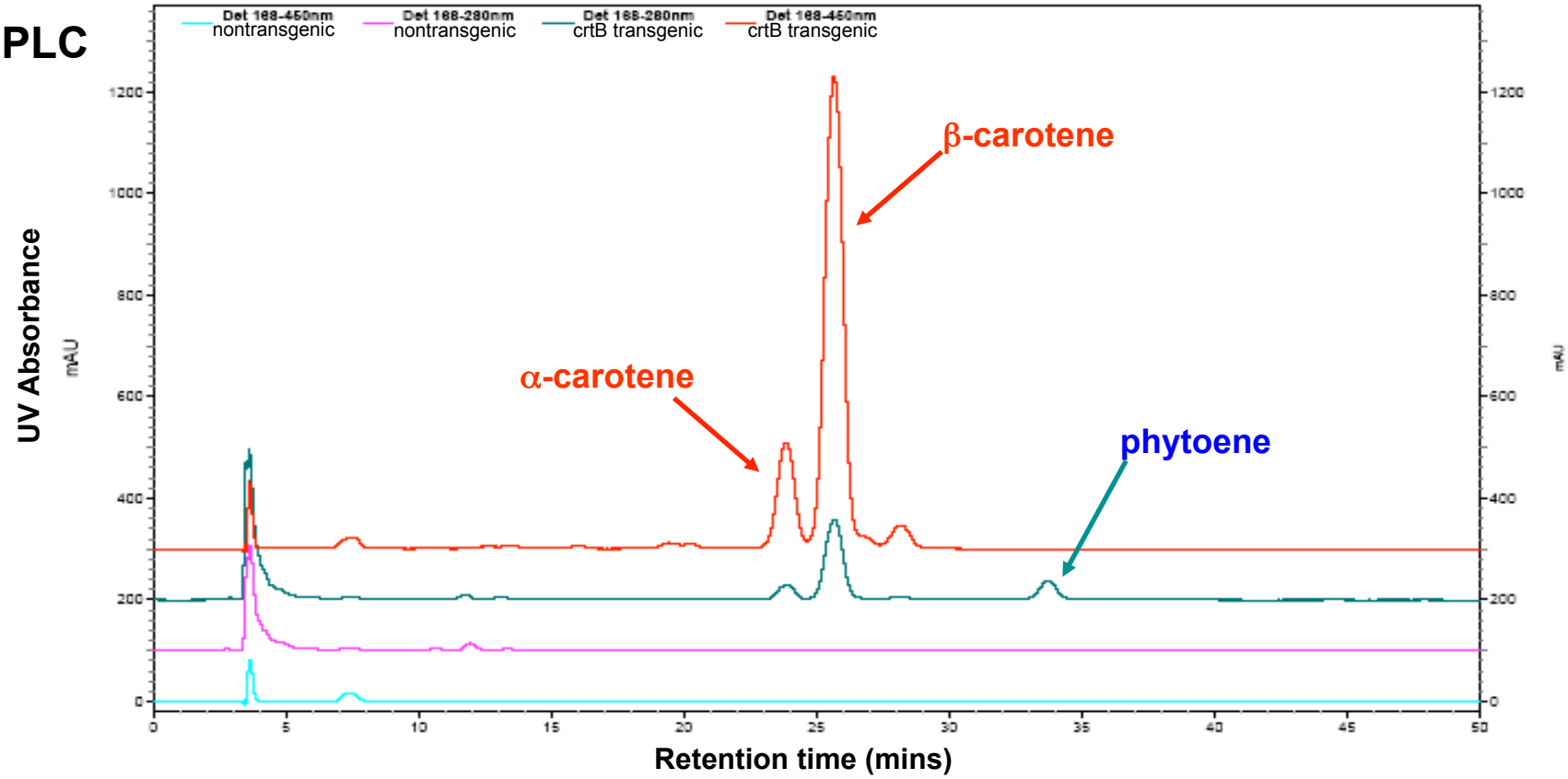


No loss of viability or yield

Good for identity preservation

Carotenoids in Soybean Seeds

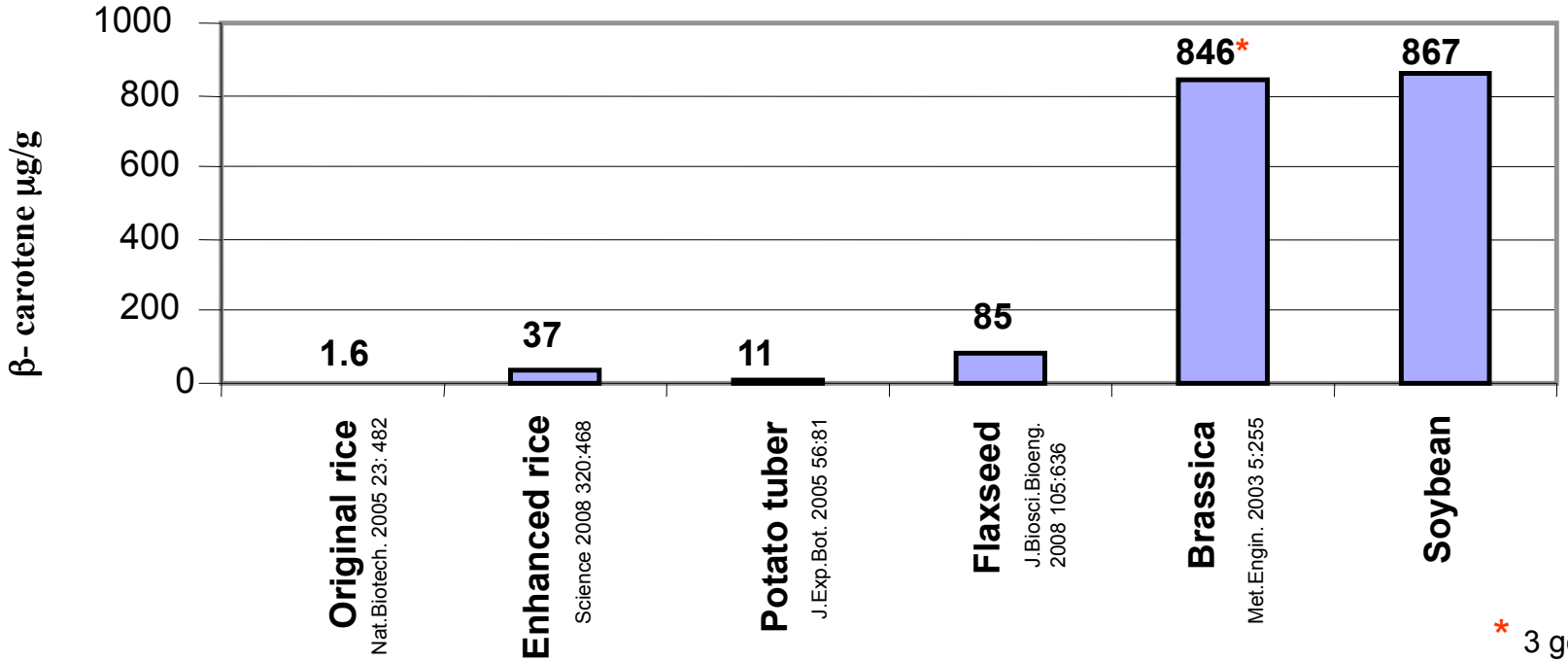
HPLC



Both α- and β-carotene are produced in transgenic soybean seeds, so other carotenoids of interest are possible (12 β: 1 α)

Pool of 'unused' phytoene in crtB transgenics may indicate higher carotenoid levels could be possible

Comparison of β-carotene in Soybean Seeds to Other Systems

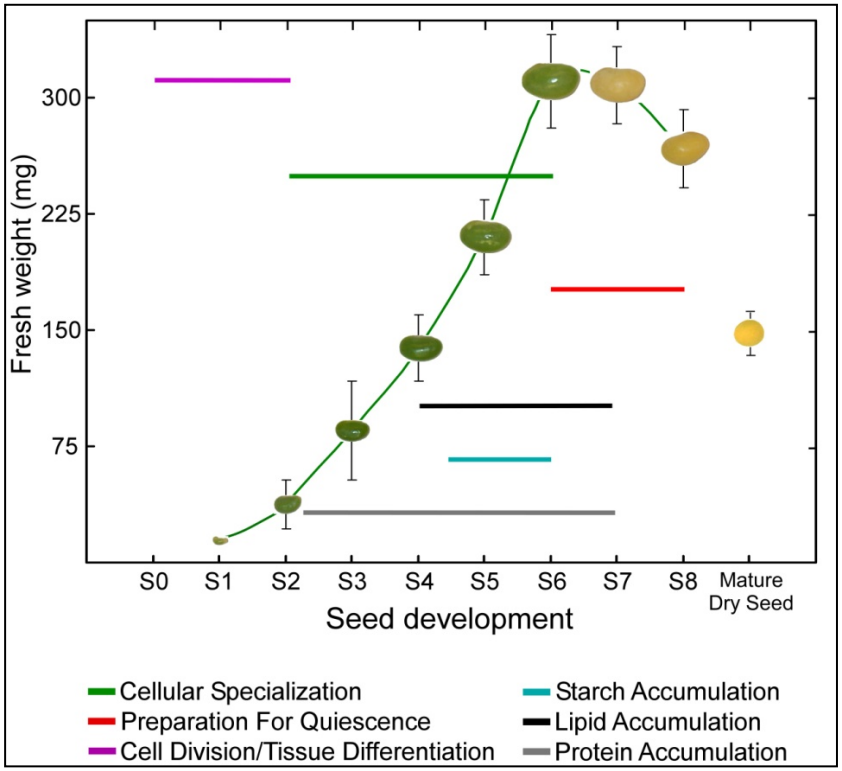


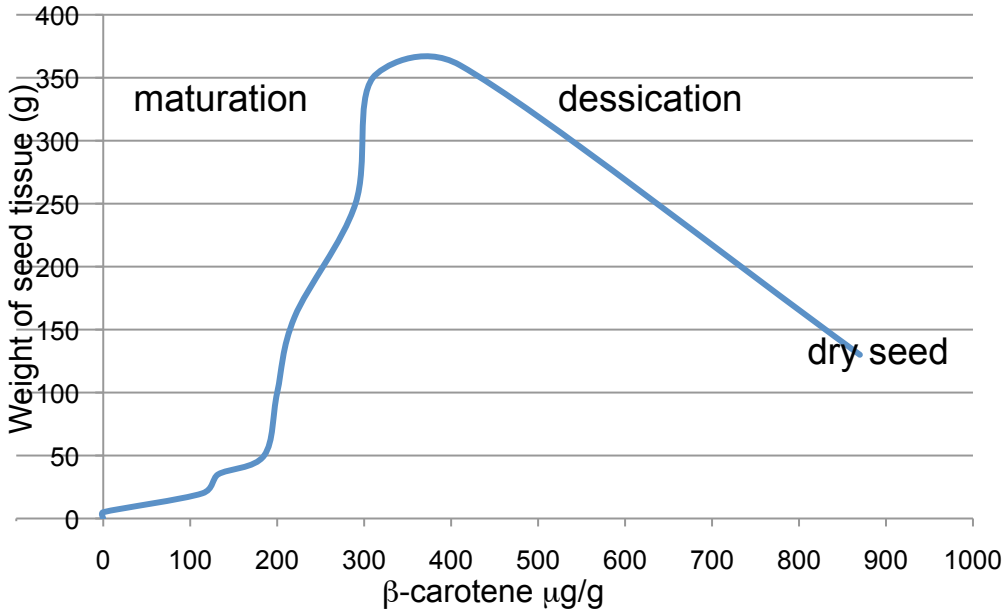
Highest level achieved in any transgenic system with a single gene

1,700-fold increase over wildtype β-carotene levels in soybean

Daily recommended dosage is 900 µg/day.
Given conversion of β-carotene to vitamin A (~20%) it would be ~ 25 seeds/day

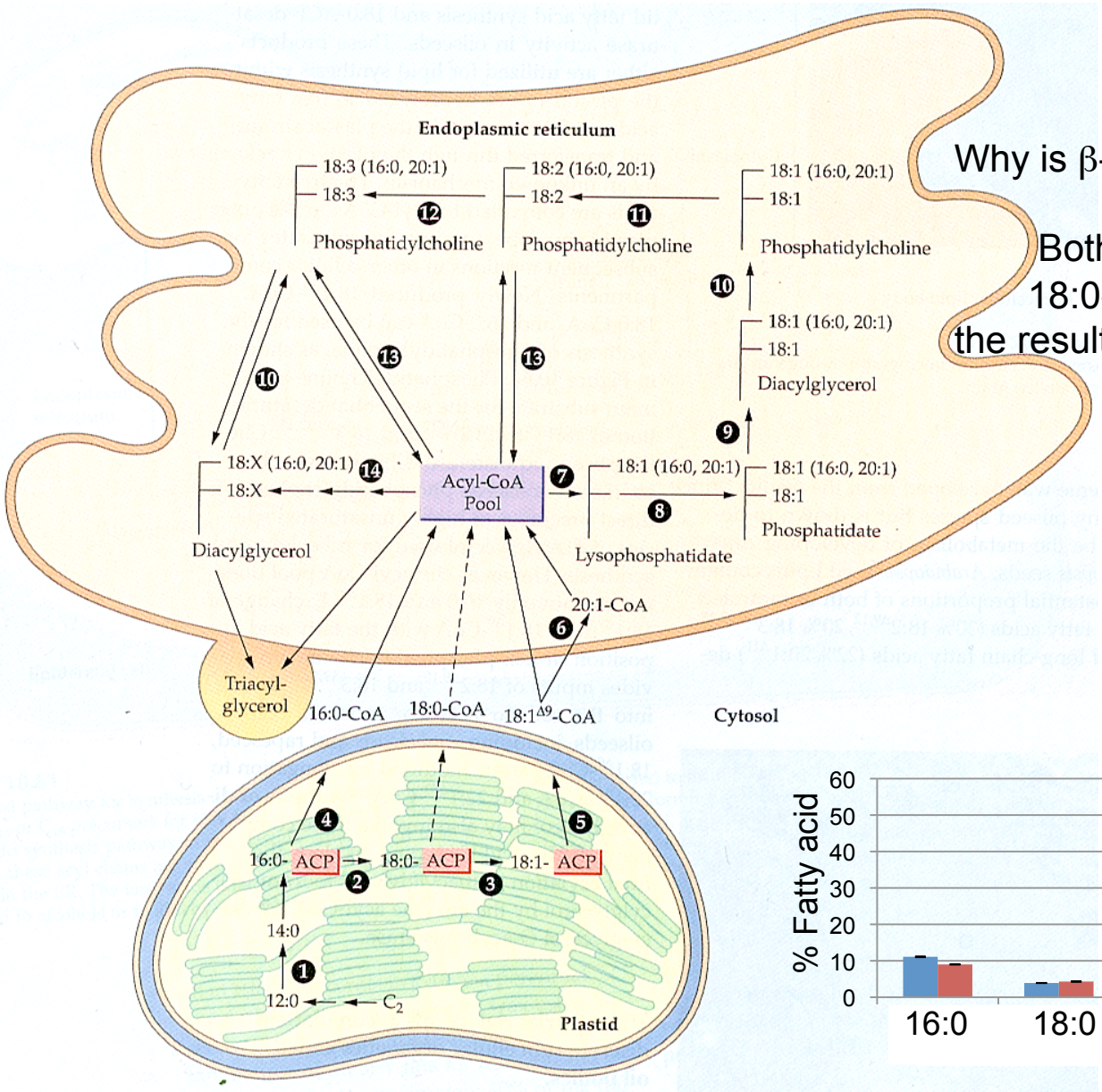
How does β-carotene accumulate during seed development?





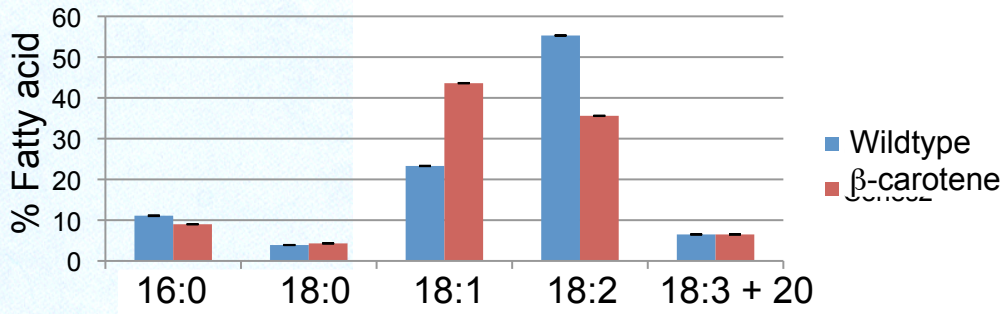
Miernyk JA, Johnston ML, Antoine W, Mooney BP, Herman EM. (2009) A Directed-Proteomics Platform for Systems Analysis of Soybean Seed Development. The Open Systems Biology Journal 2: 20-28

Did the fatty acid profile change in the crtB seeds?

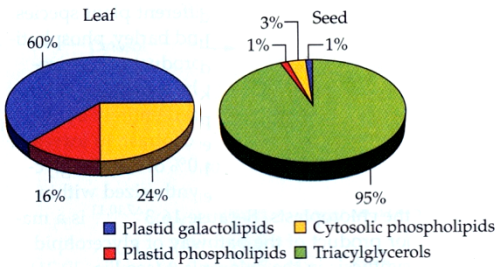


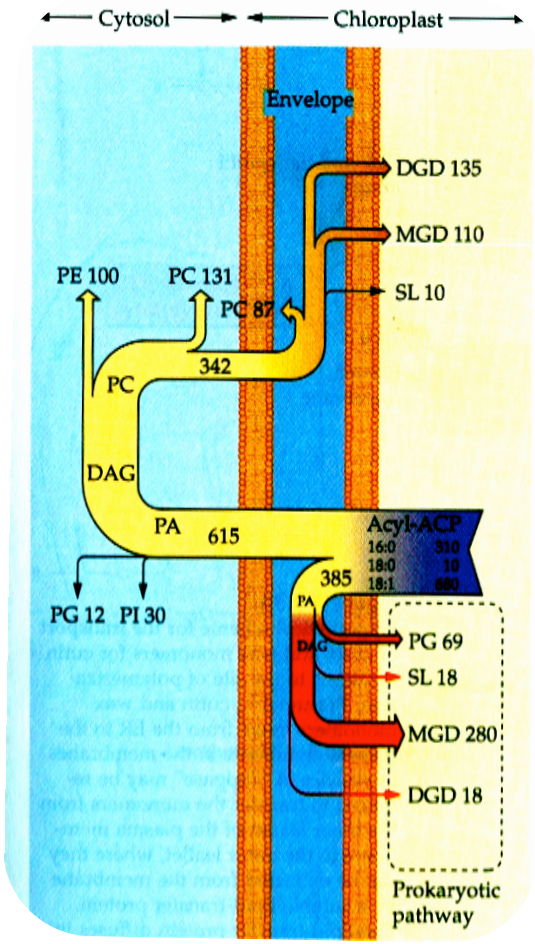
Why is β-carotene affecting 18:1 levels?

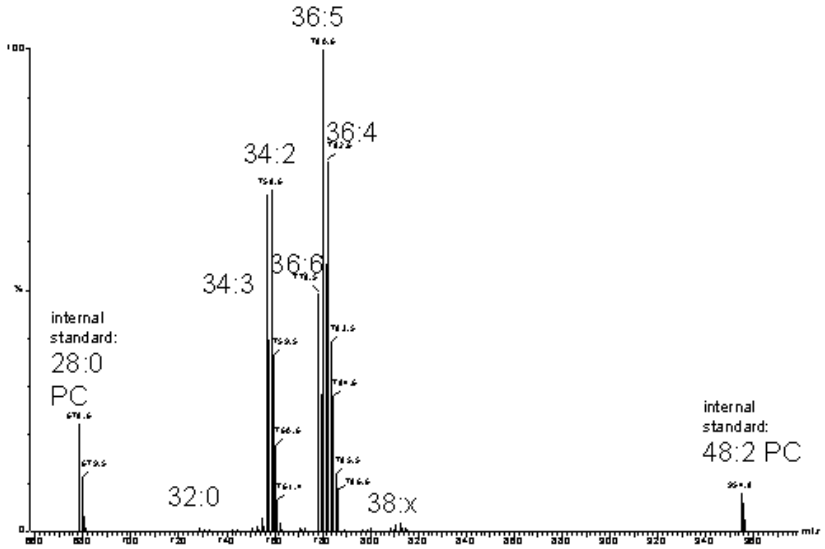
Both in plastid, might aid/stabilize 18:0-ACP desaturase activity with the result being enhanced enzyme activity

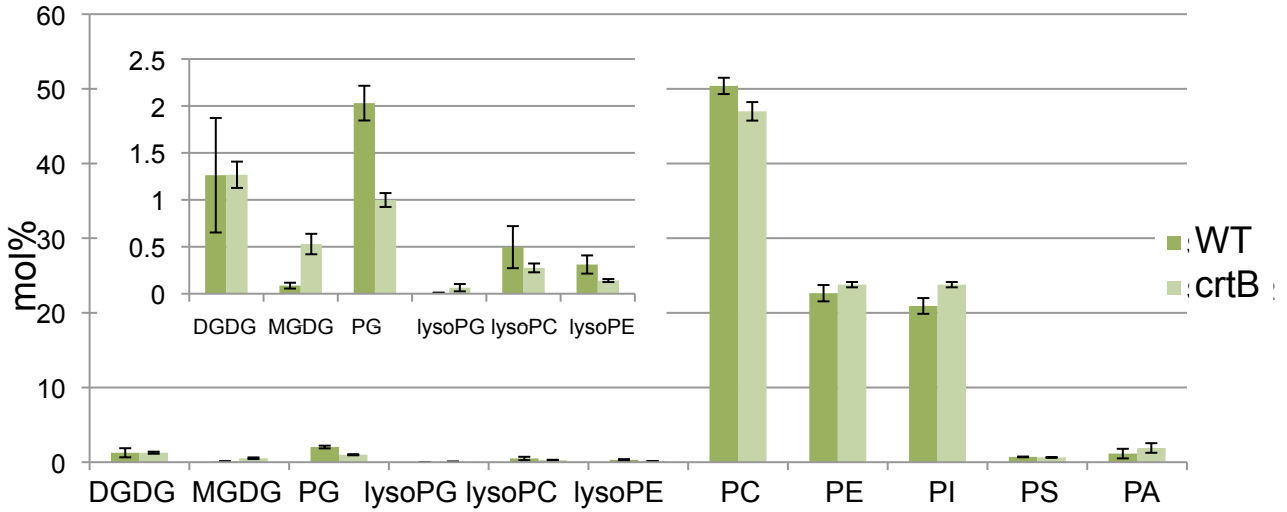


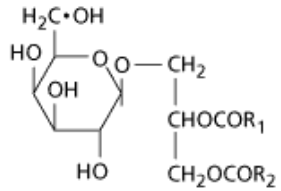
Did other lipid compounds change in the crtB seed?











Monogalactosyldiacylglycerol (MGDG)

Lipid Signaling/Lipidomics Group
Kansas State University

Bioavailablity of β-carotene in Seeds: Feeding Trials

Rainbow trout

Albino Trout

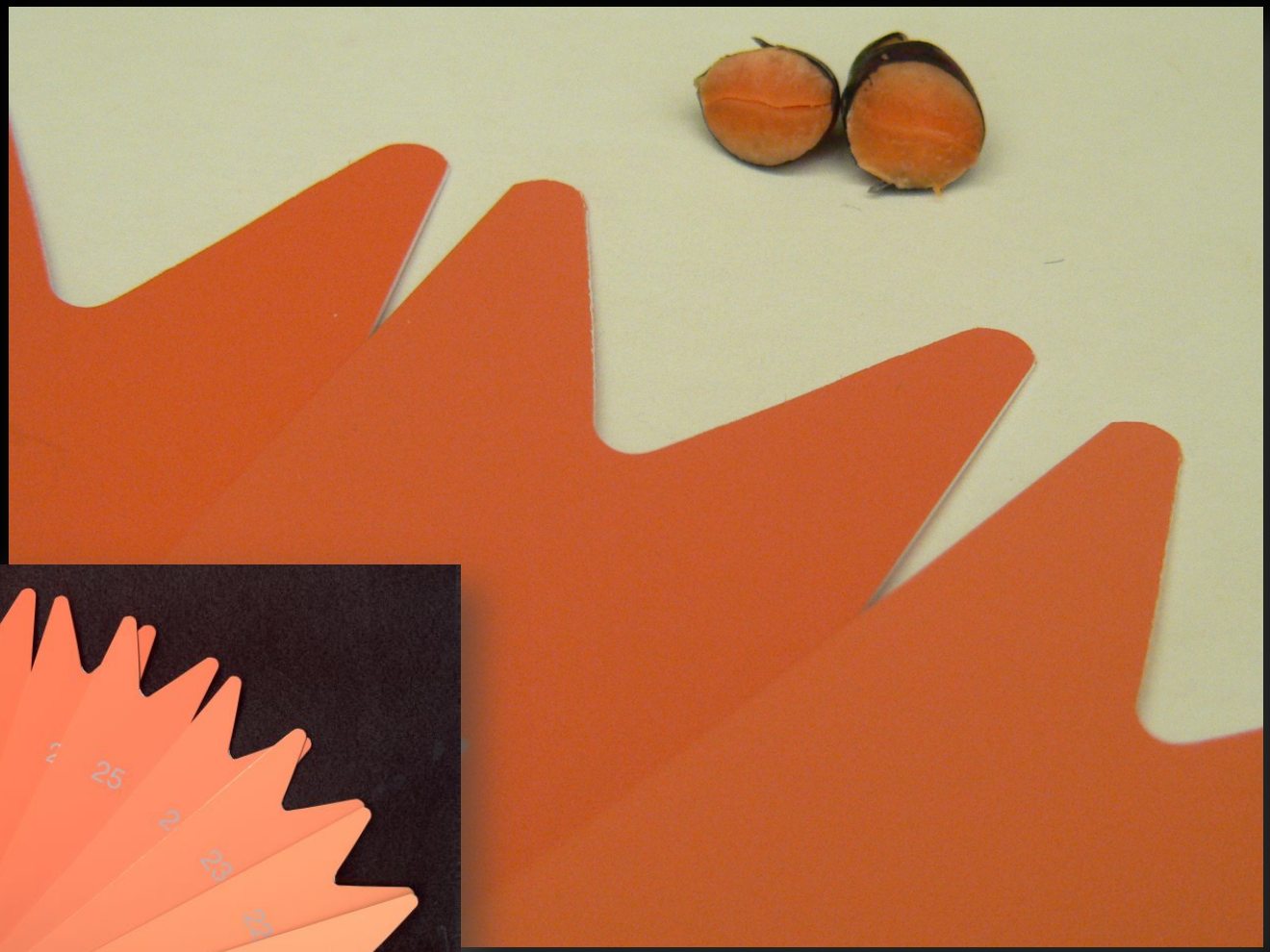
Albino Trout – ability to bio-accumulate β-carotene

Feeding trial initiated

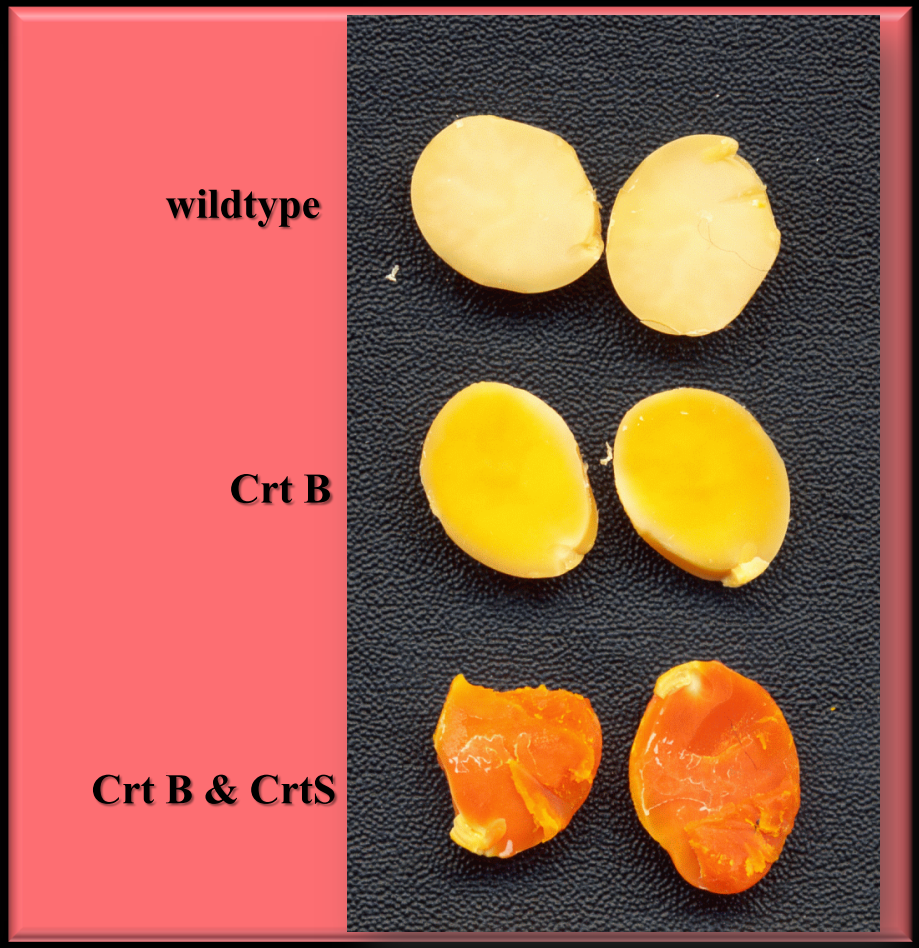
Premium for golden trout

Carotenoids in Soybean Seeds

Astaxanthin

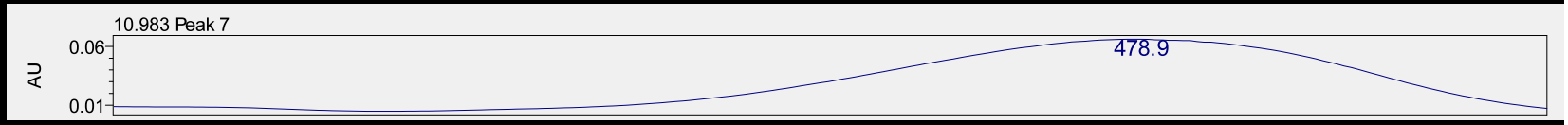


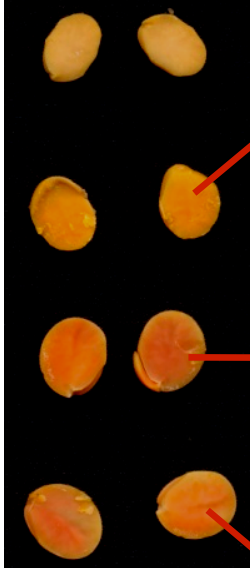
Carotenoids in Soybean Seeds

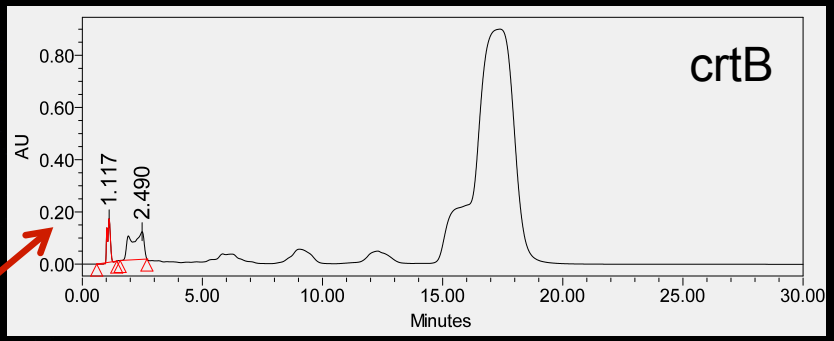


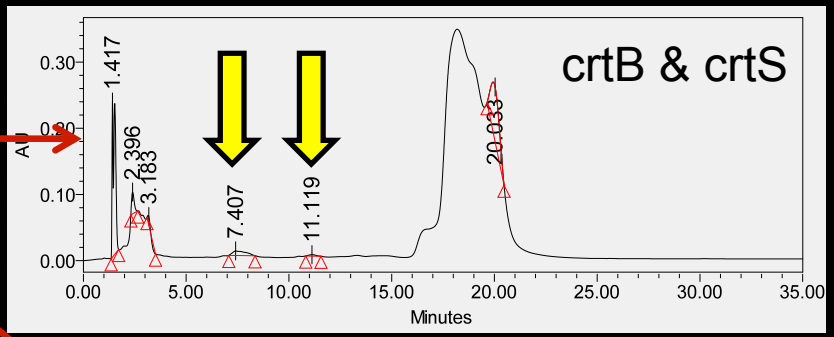
Carotenoids in Soybean Seeds

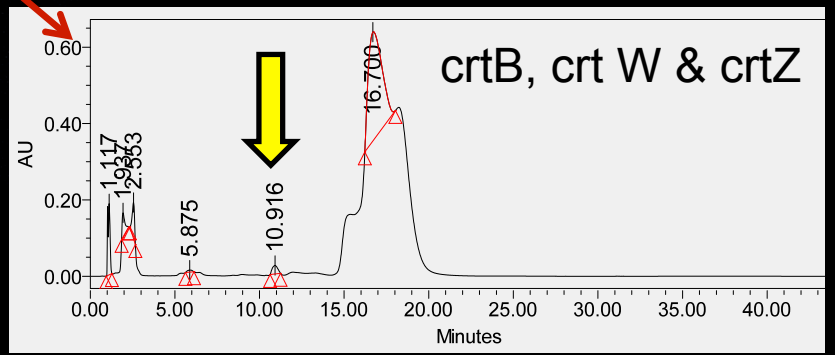
Preliminary HPLC Analysis of Astaxanthin

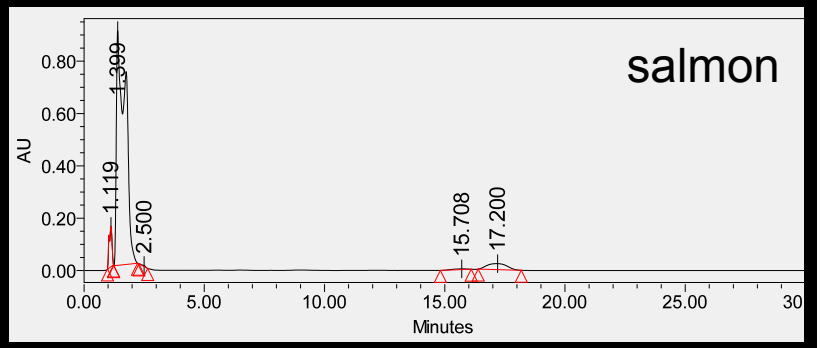


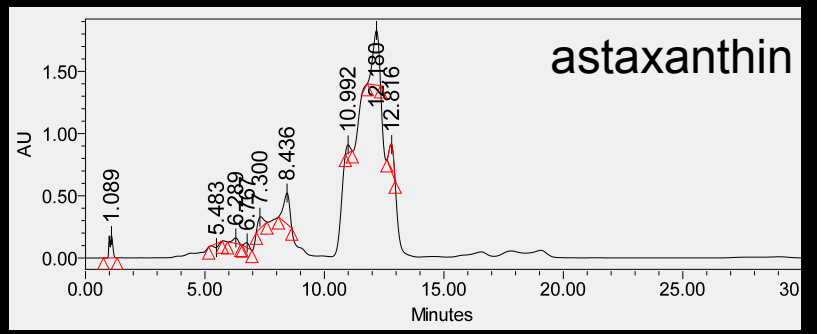












Producing Nutraceutical Carotenoids in Soybean Seeds

β-carotene

proof of concept that it is viable to express high levels of carotenoids in soybean seeds
both β and α carotene produced
β-carotene level achieved is the highest reported to date with single gene
on-going feed trails to test bioavailability
mitigating allergen sensitization

Other Carotenoids in the Pipeline

Astaxanthin for fish feed
Canthaxanthin for chick industry
Zeaxanthin for eye health

Other Crops in the Pipeline

Camelina – β-carotene for identity preservation
Peanut – β-carotene for human health

Seizing Opportunities

Many Thanks to….

Eliot Herman

Wayne Parrott
Emily Pierce

David Hildebrand

Mike Grusak

Idaho – Trout

Norway - Salmon
Chile

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