Proteomic Analysis of Developing Pecan Nuts

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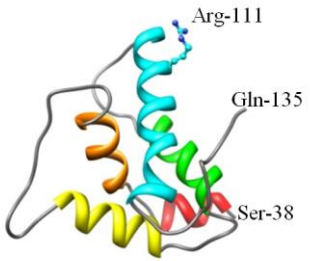
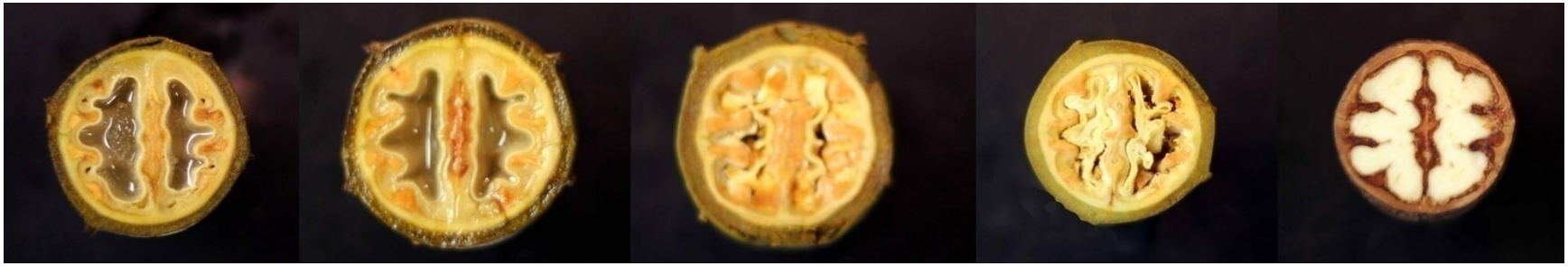




Why study proteins?

Nutritional value

Total pecan nut protein content can vary by about 30%.

- Why variation?
- Are specific proteins more variable than others?

Allergy induction

Tree nut allergies triggered by storage proteins:

- Car i 1
- Car i 2
- Car i 4

Tree's molecular toolkit

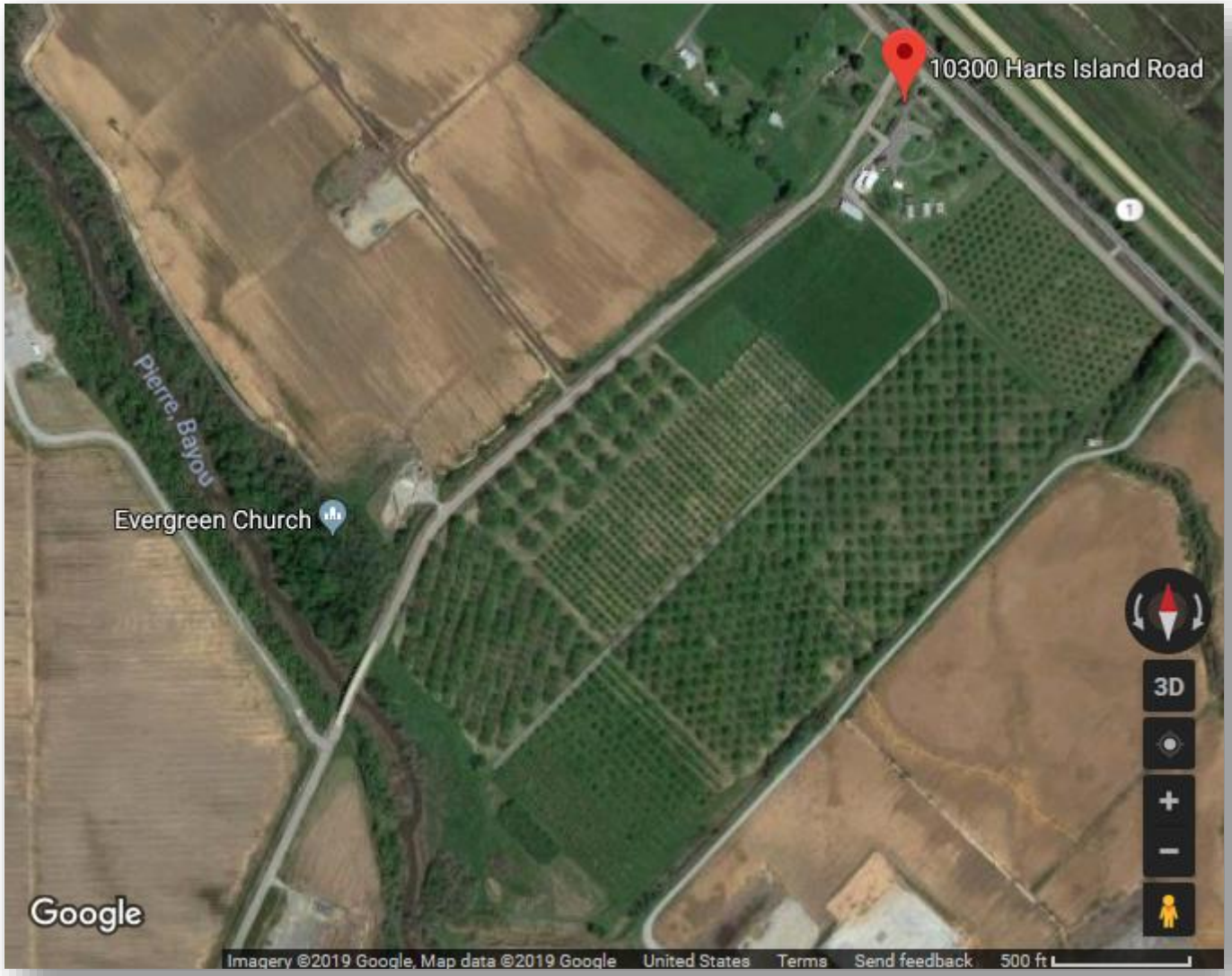
Specific proteins are molecular tools used to make other compounds in the nut:

- Oil levels, saturated vs. unsaturated oils
- Sugar metabolism

Proteomics:

Broad look at the range of proteins expressed at once in one system
(eg. a single developmental stage of pecan nut)

Samples from LSU Pecan Research and Extension Station



Pecan nut development

Water stage **Gel stage**

Aug 11

Aug 17

Aug 23

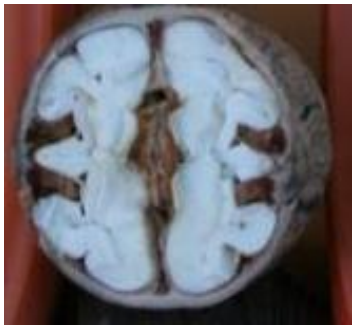
Aug 29

Dough stage **Mature stage**

Sep 4

Sep 10

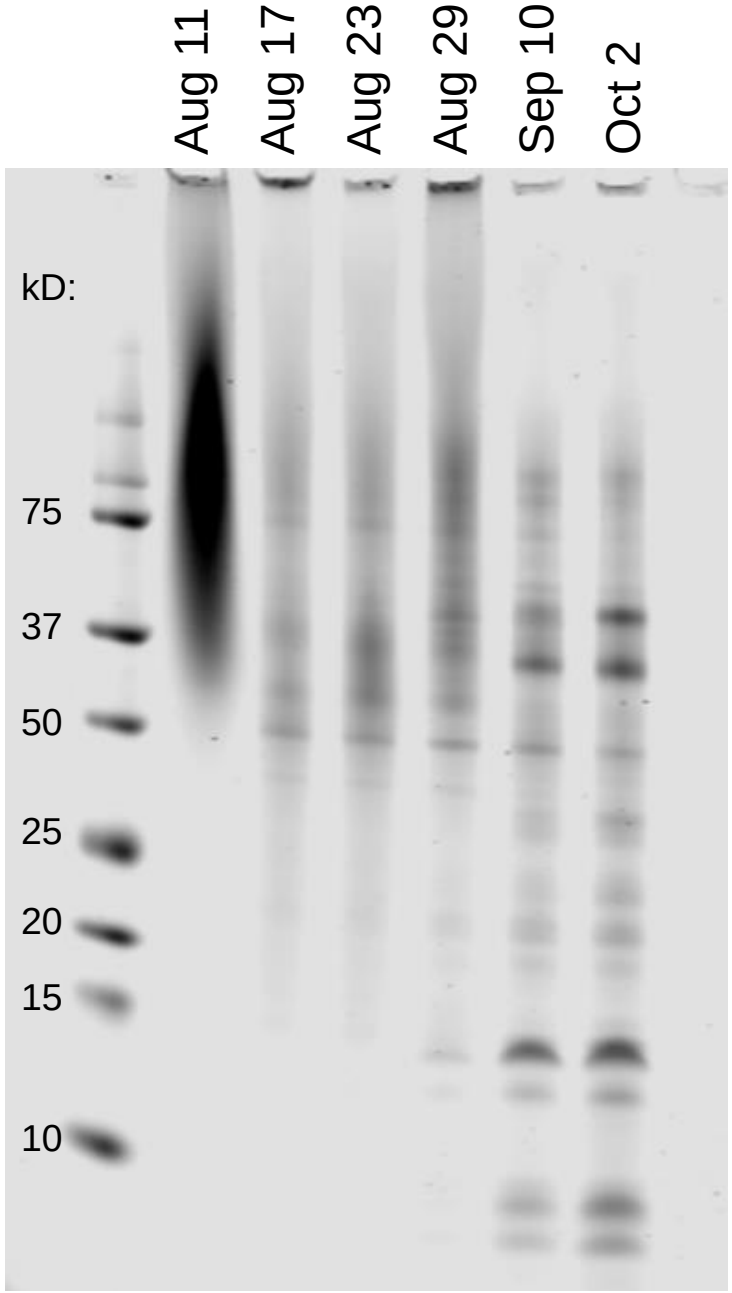
Oct 2



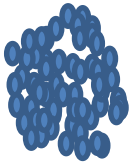
Desirable pecan nut

RNA-Seq Analysis of Developing Pecan (Carya illinoinensis) Embryos Reveals Parallel Expression Patterns among Allergen and Lipid Metabolism Genes. Mattison CP, Rai R, Settlage RE, Hinchliffe DJ, Madison C, Bland JM, Brashear S, Graham CJ, Tarver MR, Florane C, Bechtel PJ. J Agric Food Chem. 2017

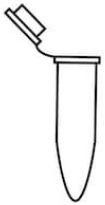
Range of proteins in nut broadens in September

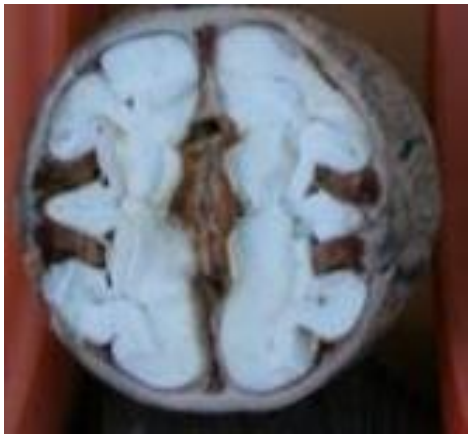


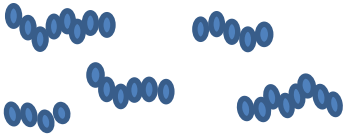
Protein identification using Mass Spec



Protein

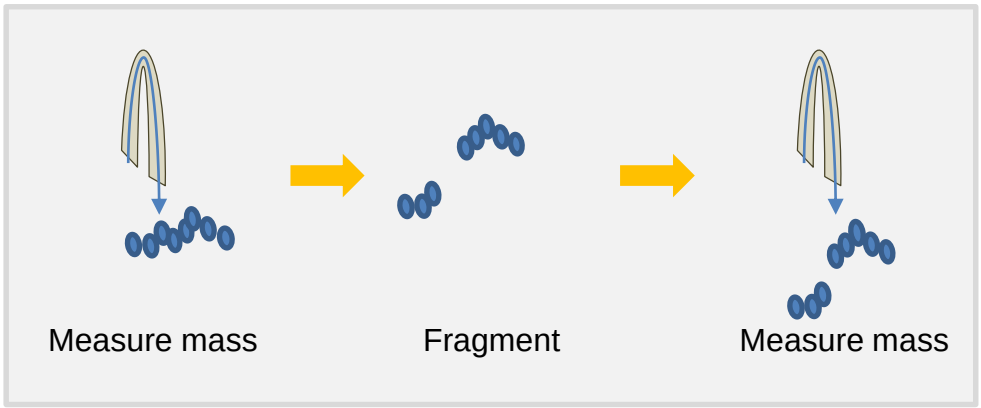




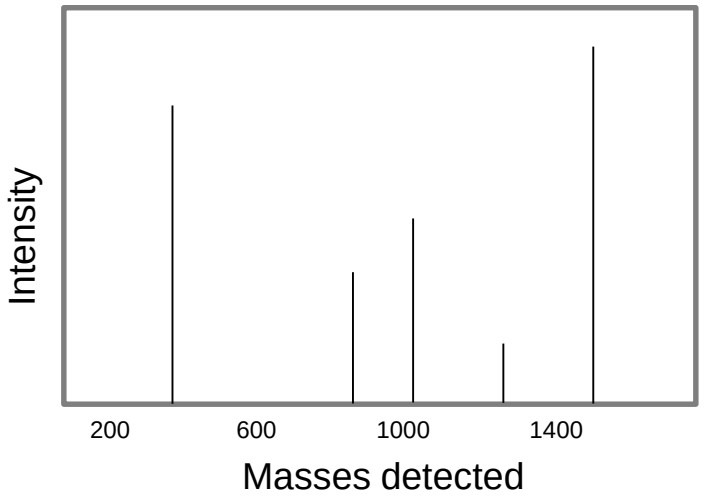


Peptide

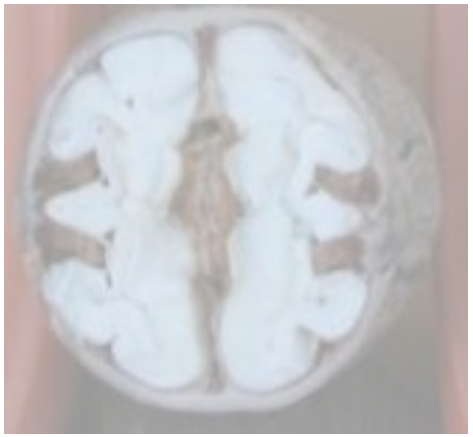
Mass spec



Read out



Protein identification using Mass Spec



Protein

Peptide

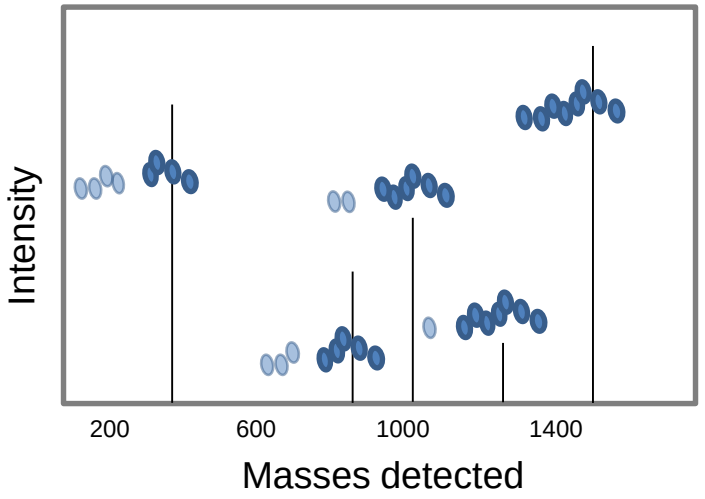
Mass spec

Measure mass

Fragment

Measure mass

Read out



Protein Mass Spec Result

Allergen: Car i 2

1	MVTKAKIPLF	LFLSALFLAL	VCSSLALETE	DLSNELNPHH	DPESHR**WEFQ**
51	**QCQER**CQHEE	RGQRQAQQCQ	RRCEEQLRER	ERER**EREEIV**	**DPR**EPRKQYE
101	QCRETCEKQD	PRQQPQCERR	CER**QFQEQQE**	**R**ERRERRRGR	DDDDKENPRD
151	PREQYRQCEE	HCRRQGQGQR	QQQQCQSRCE	ERLEEEQRKQ	EERERRRGRD
201	EDDQNPRDPE	QRYEQCQQQC	ERQRR**GQEQQ**	**LCR**RRCEQQR	QQEERERQRG
251	RDR**QDPQQQY**	**HR**CQRR**CQTQ**	**EQSPER**QRQC	QQRCERQYKE	QQGREWGPDQ
301	ASPRRESRGR	EEEQQR**HNPY**	**YFHSQGLR**SR	HESGEGEVKY	LERFTERTEL
351	LRGIENYR**VV**	**ILEANPNTFV**	**LPYHKDAESV**	**IVVTR**GR**ATL**	**TFVSQERRES**
401	**FNLEYGDVIR**	**VPAGATEYVI**	**NQDSNER**LEM	VK**LLQPVNNP**	**GQFREYYAAG**
451	**AQSTESYLRV**	**FSNDILVAAL**	**NTPR**DRLER**F**	**FDQQEQR**EGV	IIRASQEKLR
501	ALSQHAMSAG	QRPWGRR**SSG**	**GPISLK**SQR**S**	**SYSNQFGQFF**	**EACPEEHRQL**
551	**QEMDVLVNYA**	**EIKRGAMMVP**	**HYNSKATVVV**	**YVVEGTGRFE**	**MACPHDVSSQ**
601	**SYEYK**GRREQ	EEEESSTGQF	QKVTARLAR**G**	**DIFVIPAGHP**	**IAITASQNEN**
651	**LRLVGFGING**	**K**NNQR**NFLAG**	**QNNIINQLER**	EAK**ELSFNMP**	**REEIEEIFER**
701	**QVESYFVPME**	**R**QSRRGQGRD	HPLASILDFA	GFF	

Red designates detected peptides

Ratios of specific proteins change during development

Aug 11

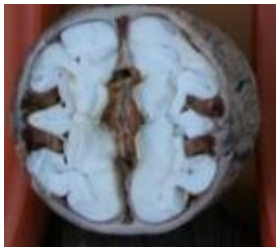
Aug 17

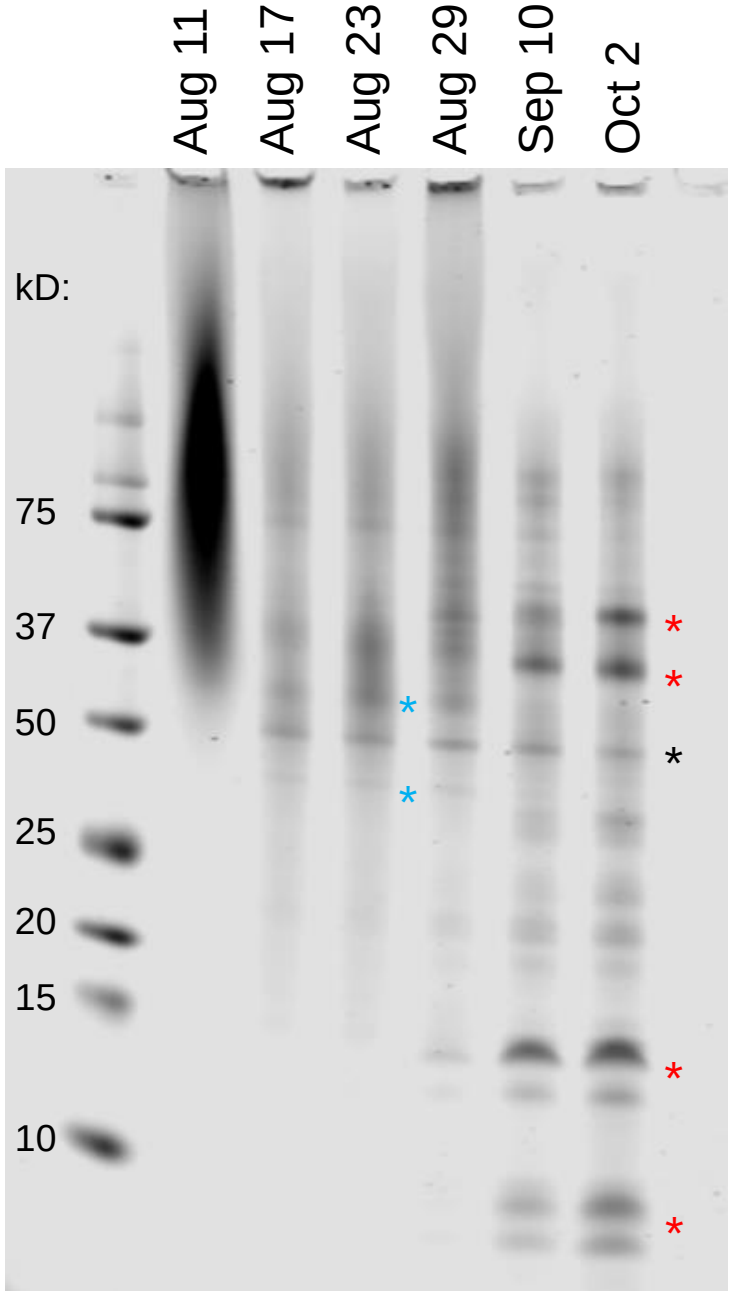
Aug 23

Aug 29

Sep 10

Oct 2





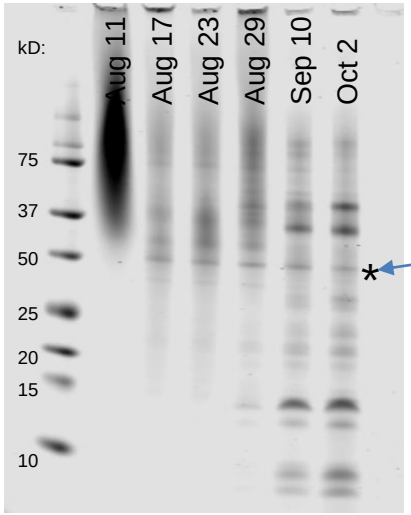
Sucrose binding protein

Glyceraldehyde -3-phosphate dehydrogenase

Protein Mass Spec Result

Glyceraldehyde-3-phosphate dehydrogenase

1	MASDKKIKIG	INGFGRIGRL	VARVVLQRND	VELVAVNDPF	INTDYMTYMF
51	KYDTVHGHWK	HHDIKVK**DSN**	**TLLFGEKAVT**	**VFGVR**NPEEI	PWGQTGAEYI
101	VESTGVFTDK	EKAAAHLKGG	AKKVIISAPS	K**DAPMFVVGV**	**NEK**EYKPELD
151	IISNASCTTN	CLAPLAKVIN	DNFGIVEGLM	TTVHSITATQ	KTVDGPSSKD
201	WRGGR**AASFN**	**IIPSSTGAAK**	AVGKVLPSLN	GKLTGMAFRV	PTVDVSVVDL
251	TVRLQKKATY	EEIKKAIKVA	SEGKLKGILG	YTEDDVVSSD	FVGDNRSSIF
301	DAKAGIALND	NFVKLVSWYD	NEWGYSTRVV	DLIVHIASVH	A



Glyceraldehyde -3-phosphate dehydrogenase

Protein identification using Mass Spec

Identified 60 hits so far

Testing parameters to get more information out of whole protein extracts

Aug 11

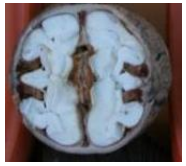
Aug 17

Aug 23

Aug 29

Sep 10

Oct 2



Proteins Identified

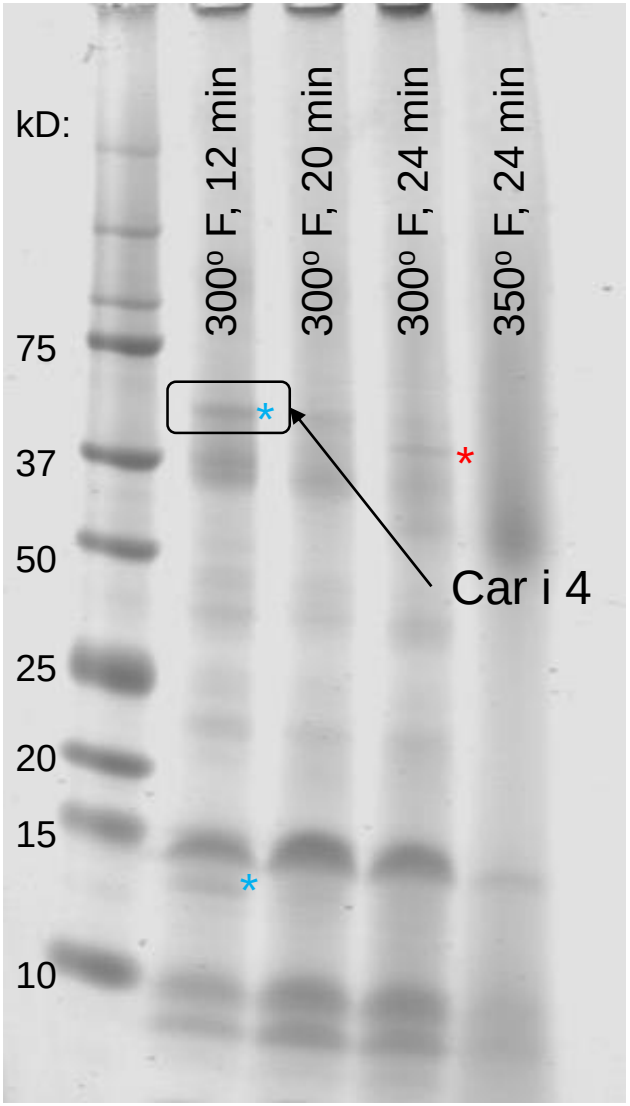
Glutaredoxin
Glutaredoxin-C1
Glyceraldehyde-3-phosphate dehydrogenase, cytosolic
Glycine-rich protein 2
IAA-amino acid hydrolase ILR1
Legumin B
Malate dehydrogenase, chloroplastic
Malate dehydrogenase, cytoplasmic
Malate dehydrogenase, mitochondrial
NADP-dependent alkenal double bond reductase P1
Non-specific lipid-transfer protein
Peroxygenase 1
Phosphoglucomutase, cytoplasmic
Phosphoglycerate kinase, cytosolic
Probable fructose-bisphosphate aldolase 3, chloroplastic
Probable nucleoredoxin 1
Protein disulfide-isomerase
Putative lactoylglutathione lyase
Pyruvate kinase, cytosolic isozyme
RuBisCO large subunit-binding protein subunit beta,
Sucrose-binding protein
Superoxide dismutase [Cu-Zn] 4A
Superoxide dismutase [Mn], mitochondrial
Thiamine thiazole synthase, chloroplastic
Translationally-controlled tumor protein homolog
Tubulin alpha chain
Universal stress protein A-like protein
Vicilin-like antimicrobial peptides 2-2

11S globulin seed storage protein 2
13S globulin basic chain
14-3-3-like protein D
18 kDa seed maturation protein
2S albumin
40S ribosomal protein S20-1
40S ribosomal protein S28
5-methyltetrahydropteroyltriglutamate--homocysteine
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
60S ribosomal protein L12
60S ribosomal protein L4
Acyl carrier protein 1, chloroplastic
Calreticulin
Chaperone protein YajL
Chaperonin 60 subunit beta 2, chloroplastic
Cinnamoyl-CoA reductase 1
Cyclic phosphodiesterase
DDT domain-containing protein DDB_G0282237
Dihydrolipoyl dehydrogenase
Elongation factor 1-alpha
Embryonic protein DC-8
Enolase
Enolase 1
Enoyl-[acyl-carrier-protein] reductase [NADH], chloroplastic
Formate dehydrogenase, mitochondrial
Fructokinase-2
Fructose-bisphosphate aldolase cytoplasmic isozyme
Glucose and ribitol dehydrogenase homolog 1

Roasted pecans

What changes happen to pecan proteins during roasting?

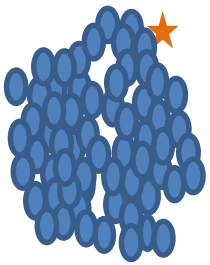
Soluble proteins



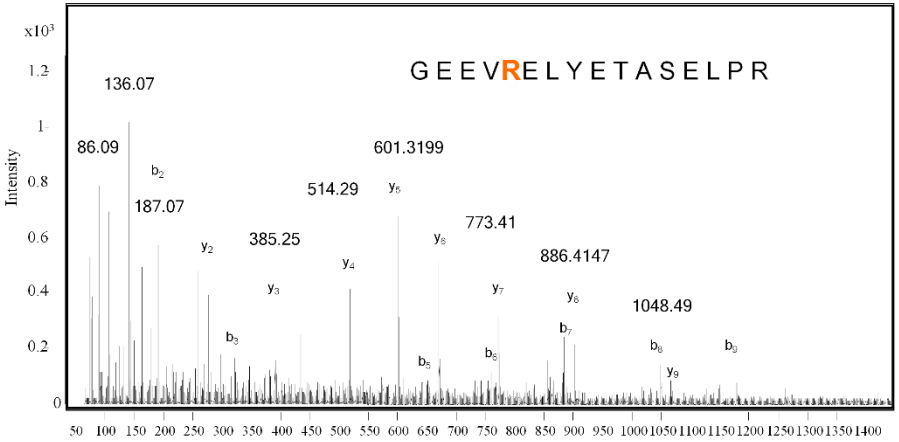
Protein modifications caused by roasting

- Protein modification is the attachment of a molecule onto a protein after it is formed
- Protein modifications seen in heated cashew nuts
- Application for roasted pecan, and pecan nut development

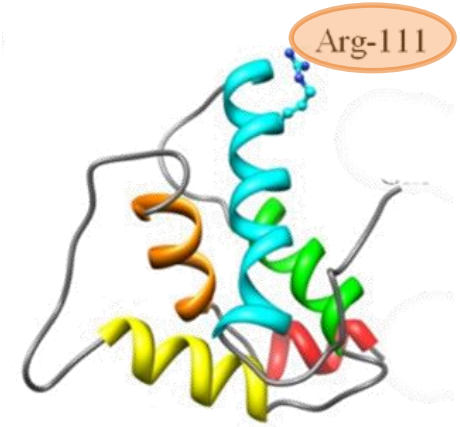
Protein modification



Cashew allergen



Protein modified cashew allergen



Identification and Characterization of Ana o 3 Modifications on Arginine-111 Residue in Heated Cashew Nuts. Mattison CP, Grimm CC, Li Y, Chial HJ, McCaslin DR, CSU, Bren-Mattison Y, Wasserman RL. J Agric Food Chem. 2017

Summary of the findings presented

What's in the pecan nut?

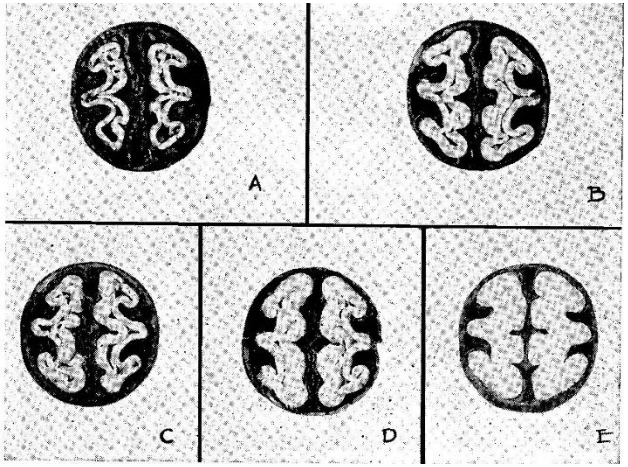
- Relative abundance of several proteins changes during development
- Relative abundance of proteins changes during heating
- Identification of these proteins via mass spec
  - Eg. Car i 4 – seed storage and allergen
  - Eg. Glyceraldehyde-3-phosphate dehydrogenase – metabolism enzyme
- Baseline of normal protein content, from which to identify variations

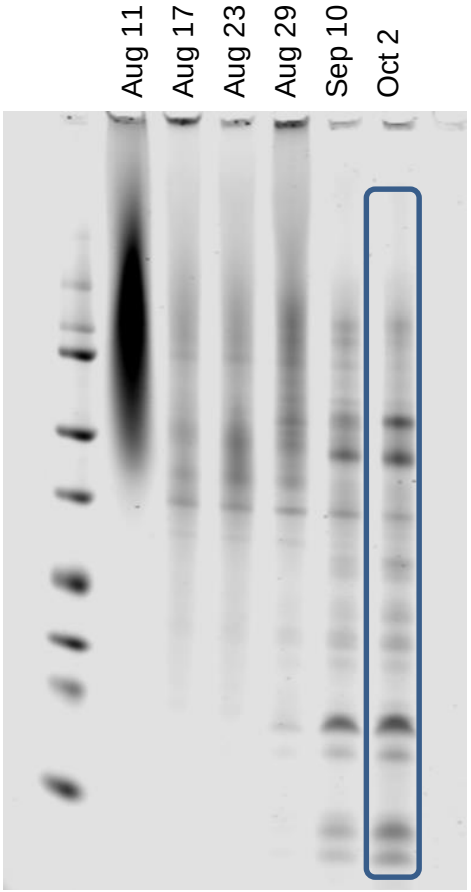
Future directions

Using list and ratios of proteins seen as a baseline

Identify differences from the baseline under:

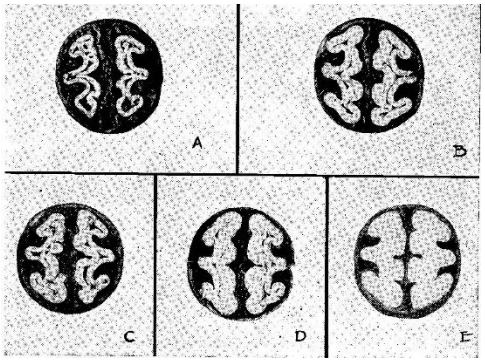
- Abiotic stress: Water, salt
- Biotic stress: Scab, stinkbugs
- Variations in allergen content





Interested in analyzing…

- Nuts aborted during development
- Nuts from disease damaged trees
  - Eg. Scab and stinkbugs
- Stressed growing conditions
  - Eg. Salt tolerance, waterlogged
- Nuts from alternate bearing trees from year to year



Potential applications

Identify protein and genetic targets

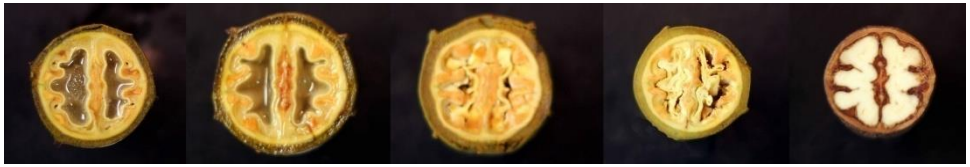
Incorporate proteins associated with important processes in the pecan nut into genetic breeding strategies

- Enable the development of new cultivars with improved sensory and nutritional quality
- Improve disease and stress tolerance
- Reduced allergen content

Thank you

Southeastern Pecan Growers Association

Chris Mattison, USDA
Charlie Graham, Noble Foundation
Casey Grimm, USDA
Steven W. Lloyd, USDA



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