

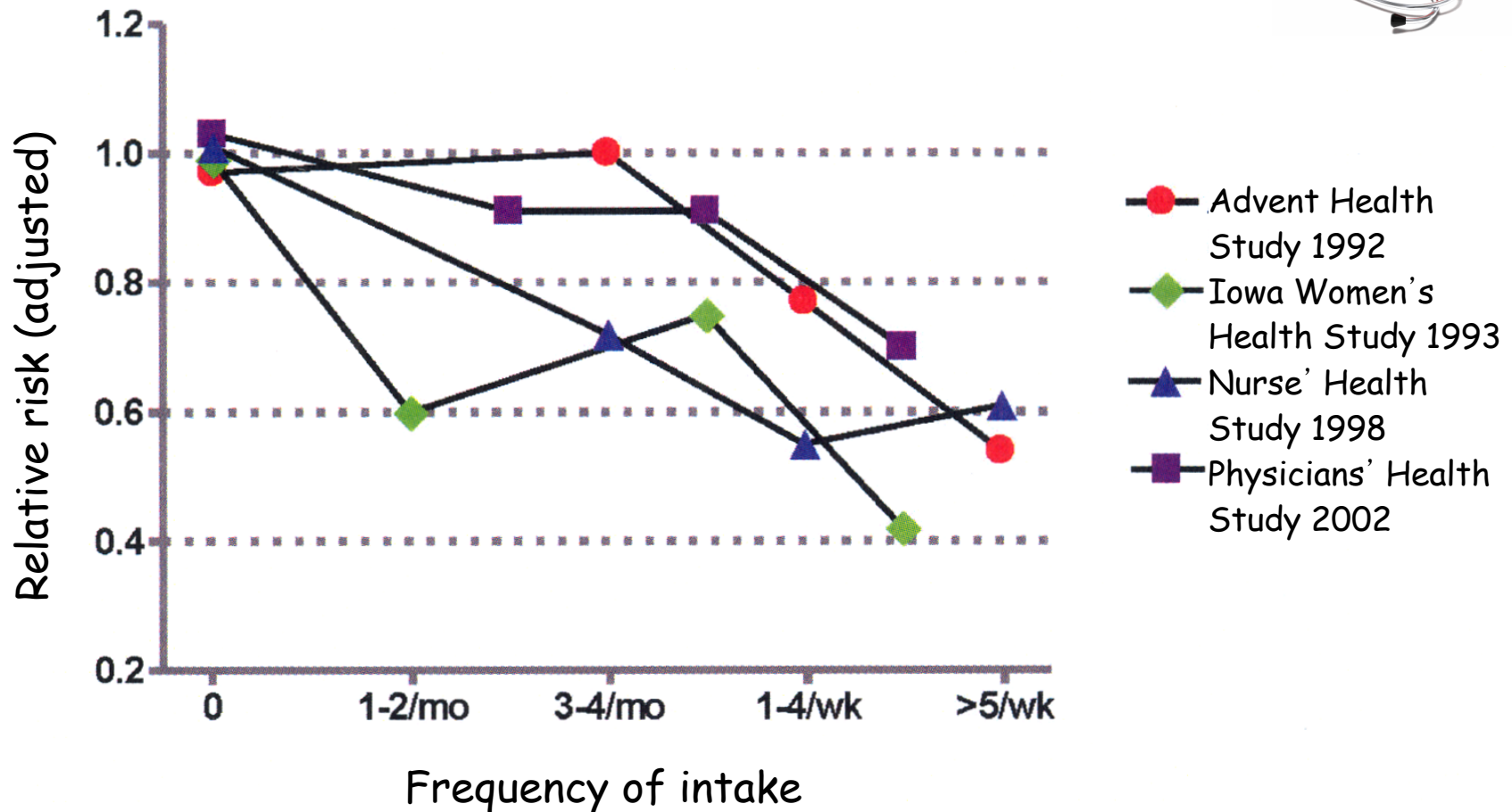


The Phytonutrients and Health Benefits of U.S. Pecan Consumption

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Pooled analysis: Subjects >5/wk had a 37% reduction in multivariable-adjusted risk of



Results of prospective studies of nut consumption and risk of death from heart disease → causal association.

Clinical Studies with Pecans



A Randomized Control Pecan Feeding Study

Morgan and Clayshulte (2000). Pecans lower low-density lipoprotein cholesterol in people with normal lipid levels. *Journal of the American Dietetic Association*, 100:312-318.

Objective - To investigate the serum lipid profiles and dietary intakes of individuals with normal lipid levels who consumed pecans and those who did not.

Intervention - Pecans, 68 g/day for 8 weeks, plus "self-selected" diets, whereas the control group avoided all nuts and consumed "self-selected" diets.

Although there is variability in the dietary habits of food choices made & calories consumed, this approach represents perhaps a “realistic appraisal” of the impact of the pecan set forth in the FDA qualified health claim.

Results

- LDL (or bad) cholesterol was lowered in the pecan treatment group from 2.61 ± 0.49 mmol/L to 2.35 ± 0.49 mmol/L, after 4 wk whereas in the control group, LDL cholesterol levels increased to 3.03 ± 0.57 mmol/L.
- Dietary fat, MUFA, PUFA, insoluble fiber, Mg, and energy were significantly higher in the pecan treatment group than the control group.



Single-blind, Randomized, Controlled, Crossover Pecan Feeding Study

Rajaram *et al.* (2001). A monounsaturated fatty acid-rich pecan-enriched diet favorably alters the serum lipid profile of healthy men and women. *The Journal of Nutrition*, **131**:2275-2279.

Objective - To investigate the effect of pecans on blood lipids & lipoproteins in healthy men & women compared to the Step I diet of the National Cholesterol Education Program.

Approach - Diets were made isoenergetic by reducing all items in the Step I diet by 20% & replacing the calories with pecans - 72 g/day.

Serum lipid, lipoprotein and apolipoprotein (apo) []s in men & women who consumed Step I and pecan-enriched diets

	Baseline values	Step I Diet	Pecan Diet	% Change
Total cholesterol, mmol/L	5.04 ±	4.78 ±	4.47 ±	-6.7
LDL cholesterol, mmol/L	0.84	0.75	0.70	-10.4
HDL cholesterol, mmol/L	3.27 ±	3.05 ±	2.73 ±	+5.6
LDL : HDL cholesterol	0.65	0.56	0.51	-15.7
TAG, mmol/L	1.14 ± 0.26	1.20 ±	1.21 ± 0.25	-11.1
Apo A1, g/L	2.86 ±	0.23	2.37 ±	+2.2
Apo B, g/L	0.90	2.81 ±	0.70	-11.6
Lipoprotein (a), g/L	1.23 ±	0.90	1.16 ± 0.69	-15.1
	0.67	1.29 ±	1.36 ± 0.21	
	1.33 ±	0.77	0.75 ±	-23
	0.20	1.30 ±	0.19	

Rajaram et al. (2001). Values are means ± SD, n = 23



A Qualified Health Claim



- Heart disease remains the #1 killer in the USA. Heart Disease and Stroke Statistics for 2012 from the AHA list CVD as being responsible for 32.8% of all deaths nationwide in 2008.
 - In July 2003, the US Food & Drug Administration approved the 1st qualified health claim for tree nuts.
- “Scientific evidence suggests, **but does not prove**, that eating 1.5 ounces per day of some nuts, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.”*



Almonds
20-24

What is an ounce of tree nuts?
The answer varies depending on the type and size of each nut.



Cashews
16-18

Here are examples of one-ounce portions of each tree nut, along with the average number of nuts per serving.



Macadamias
10-12



Brazil Nuts
6-8

Nutrition in Every Handful



Hazelnuts
18-20



Pecans
18-20
HALVES



Pine Nuts
150-157



Pistachios
47-49



Walnuts
10-14
HALVES

Source: INC and USDA National Nutrient Data Base for Standard Reference, Release 19, 2006. 8/07



2011 Loma Linda Study



- Pecan consumption increased plasma postprandial antioxidant capacity & catechin levels, and decreased LDL oxidation in humans.
- A randomized, placebo-controlled, crossover trial with a 1-wk washout period between treatments (whole pecans, blended pecans, or control) showed that γ -tocopherol levels 2x at 8 h, and $H\text{-ORAC}_{FL}$ and $L\text{-ORAC}_{FL}$ values increased by 12 and 10%, respectively, at 2 h.
- After pecan consumption, oxidized LDL decreased by 30, 33, and 26% at 2, 3, and 8 h, respectively, while PAC monomer levels at 1 and 2 h were higher than at baseline (0 h) and after the control test meal.



2011 Loma Linda Study



- **Conclusions** ... these results show that bioactive constituents of pecans are absorbable and contribute to postprandial antioxidant defenses in the human body.
- The phenolic composition of pecans is complex and largely unknown. When pecans are consumed, their catechin monomers (*i.e.*, the flavan-3-ols; building blocks of the proanthocyanidins [PACs]) are absorbed, but more knowledge on the antioxidants in pecans is warranted.

Hudthagonsol *et al.* 2011. Pecans acutely increase plasma postprandial antioxidant capacity and catechins and decrease LDL oxidation in humans. *The Journal of Nutrition* 141: 56-62.



The Nutrients

PECAN NUTRITION FACTS

SERVING SIZE
1 OZ.(28.35G) APPROX 19 HALVES

Amount Per Serving

Calories	200	Calories from Fat	180
		%Daily Value*	
Total Fat	20g		
Saturated Fat	2g		
Polyunsaturated Fat	6g		
Monounsaturated Fat	12g		
Cholesterol	0mg	0%	
Sodium	0mg	0%	
Potassium	116mg	4%	
Total Carbohydrate	4g		
Dietary Fiber	3g		
Protein	3g		
Vitamin A	0%	Vitamin C	0%
Calcium	2%	Iron	4%
Vitamin E	2%	Thiamin	10%
Vitamin B ₆	2%	Folate	2%
Phosphorus	8%	Magnesium	8%
Zinc	8%	Selenium	2%
Copper	15%	Manganese	60%

*Percent Daily Values are based on a 2,000 calorie diet. Daily Values may be higher or lower depending on your calorie needs. Data from the USDA National Nutrient Database For Standard Reference Release 22 (2009).

**Pecans nuts are unsalted and unroasted.



Proximates for Stuart Pecans¹



	USDA (%)	Experimental ¹ (%)
Moisture	3.52 ± 0.11	4.97 ± 0.02
Total lipid	71.97 ± 0.12	70.30 ± 0.57
Carbohydrate	13.86	14.17
Fiber, TD	9.60 ± 0.41	9.41 ± 0.49
Protein	9.17 ± 0.09	9.11 ± 0.10
Ash	1.49 ± 0.06	1.45 ± 0.05

USDA National Nutrient Database for Standard Reference, Release 28, 2015

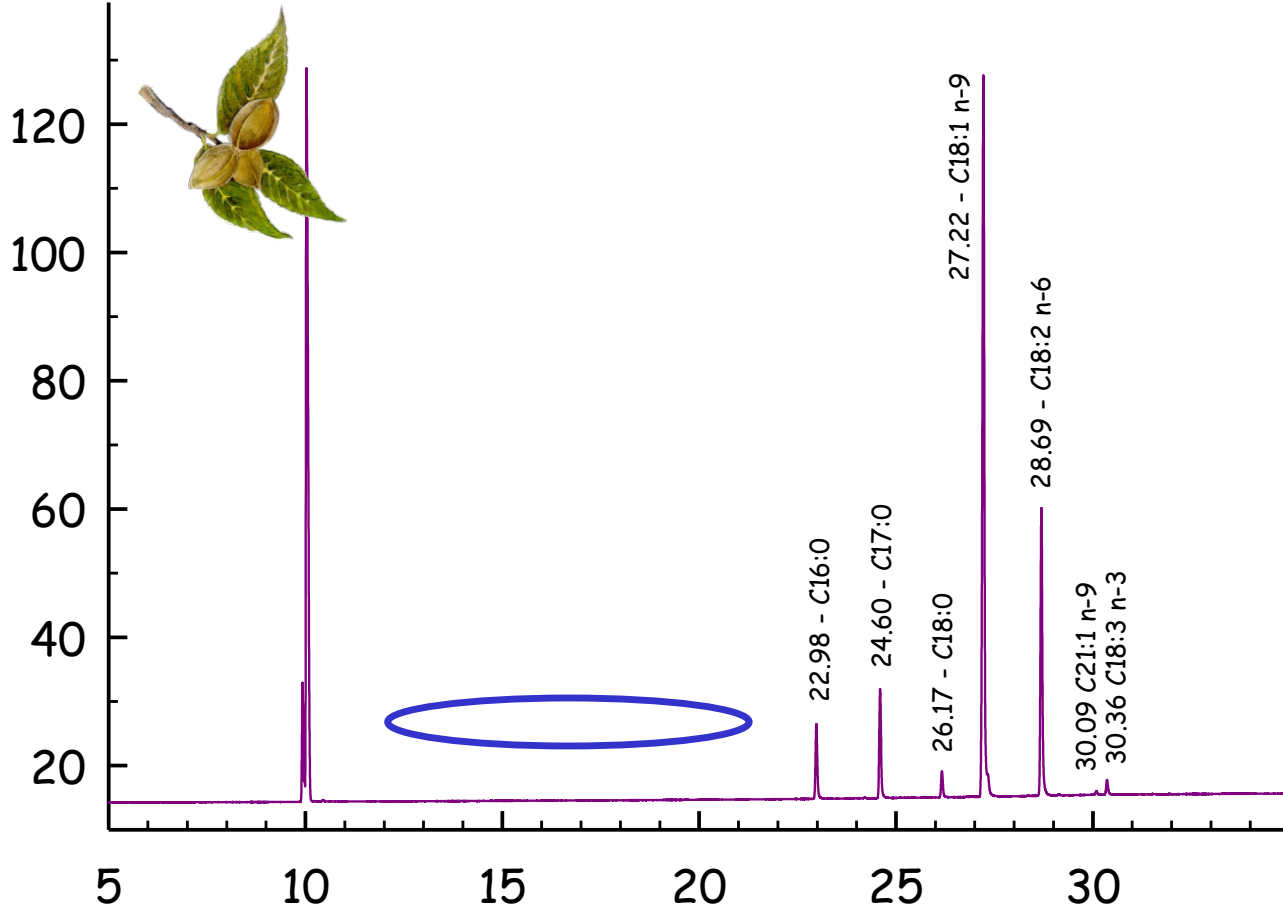


The Good Fats in Pecans

	Weight %			
	18:1	18:2	18:3	Saturates
Pecan	67	22	1	10
Olive Oil	70	16	-	14
	MUFA	PUFA	PUFA	SFA



FID Signal Intensity



Retention time, min

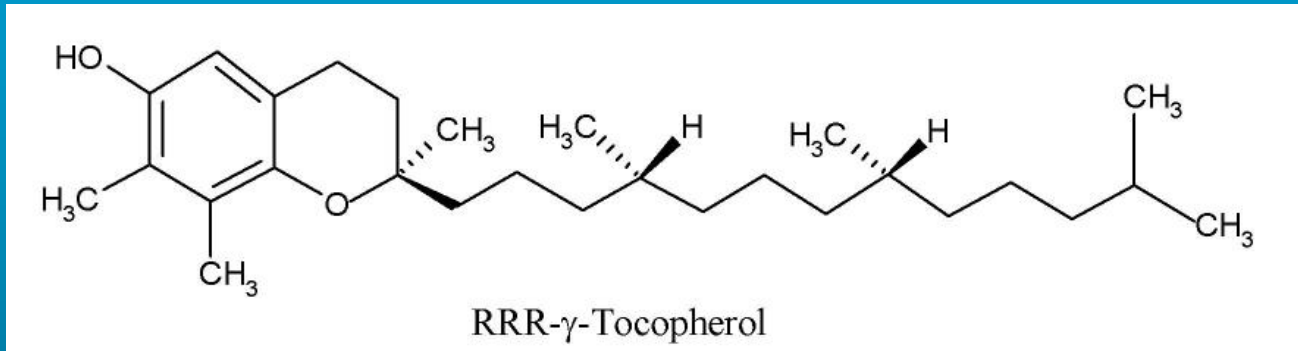
C16:0	C18:0	C18:1 (n-9)	C18:2 (n-6)	C18:3 (n-3)	C21:1 (n-9)
6.10	2.36	67.09	22.76	1.15	0.37

Vitamin E in Tree Nuts & Peanuts (mg/100 g)

	α -T	γ -T	Total
Pecans*	1.4	30.4	31.9
Peanuts, Raw**	11.0	10.3	22.5
Dry Roasted	4.1	6.2	11.3
Peanut Butter	10.2	9.7	20.1
Almonds*	29.5	1.0	29.5
Pistachios*	2.3	25.1	29.7
Walnuts, English*	1.2	25.5	30.5
Cashews*	1.0	4.5	5.9
Macadamias	--	--	1.8

*UGA data, Lee et al. 1988; **UGA data, Chun et al., 2003.

Potential Role of γ -Tocopherol



- A large amount of ingested γ -tocopherol is excreted. It has a role in the body as a detoxifying agent (*i.e.*, against free radicals, carcinogens).
- Efficient antioxidant for inactivation of nitric oxide species and other reactive nitrogen species (RNS).
- Understanding of the role of γ -tocopherol in human health is still developing.

Arginine Content of Selected Foods

(mg arginine/g protein)

Tree Nuts

Macadamia	177
Brazil Nuts	150
Pecans	128
Almonds	116
Pistachios	98
Peanuts	119

Vegetables

Broccoli	67
Spinach	57
Lettuce, green leaf	52
Potatoes, Irish	49
Bean, green	40

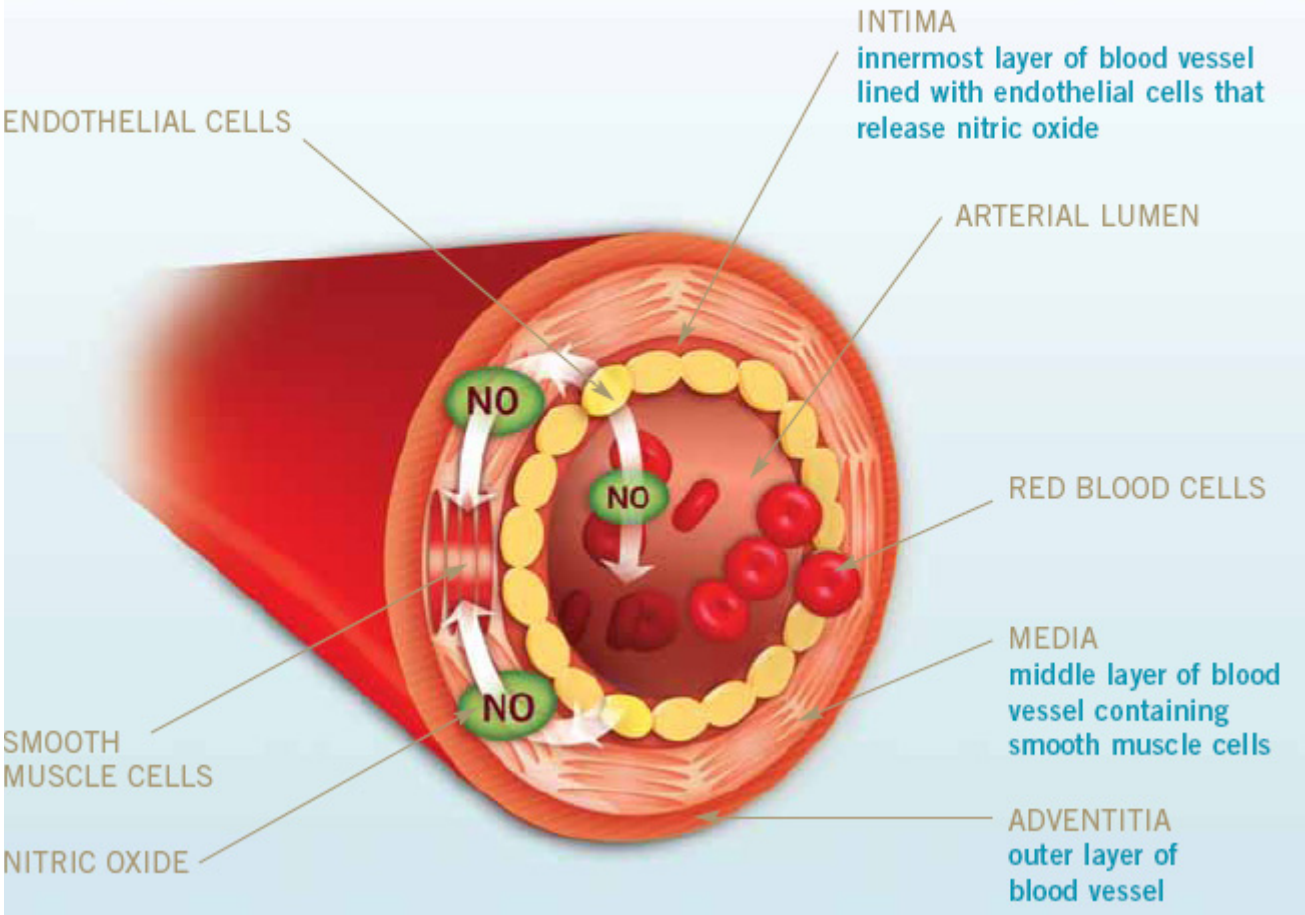
Meats and Fish

Beef, round	63
Chicken	63
Salmon	60
Eggs	65
Milk, Whole	23

Fruit

Grapes	67
Orange Juice	63
Apples	23
Soybeans	78

* USDA Database, Release 28, 2015

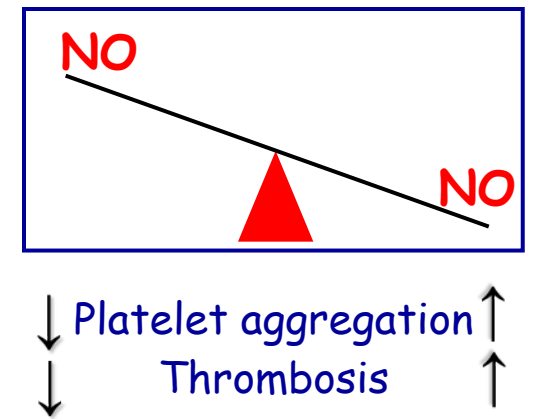


Vascular health

- L-arginine
- L-glutamate
- L-glutamine
- L-citrulline
- glycine

Vasculopathy

- L-lysine
- L-methionine
- homocysteine



Pecan consumption can help promote vasodilation!

Adapted from Huynh & Chin-Dusting, 2006



Phytochemicals





Antioxidants

•In plants ...

the antioxidants acts as phyto-protectants.

•In foods ...

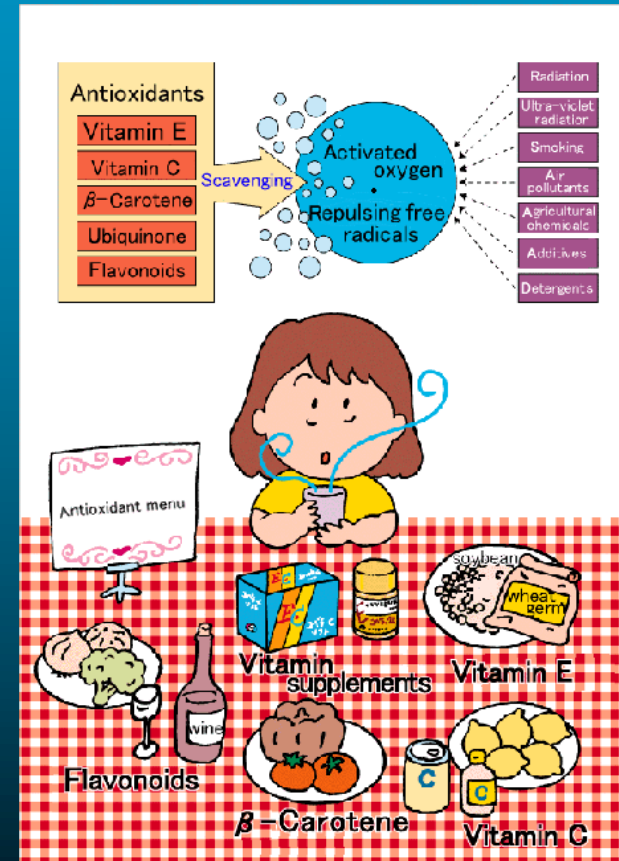
the antioxidants protect lipids from oxidation.

may also help preserve color.

•In humans and animals ...

the antioxidants may provide activity *in vivo*.

they have a solid scientific track record for positive effects on heart health.



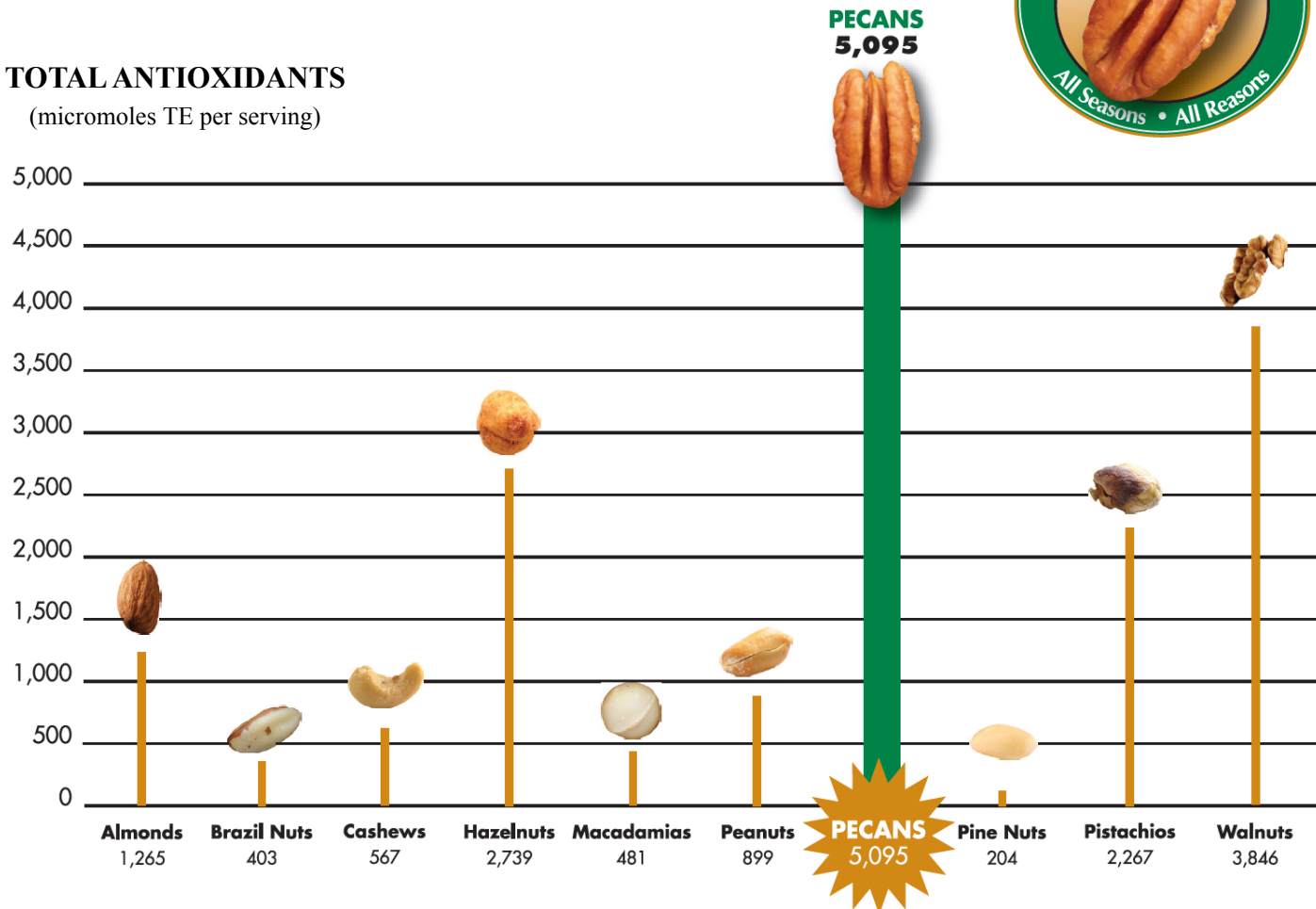
Pecans: Top Nut for Antioxidants

A USDA study finds that pecans lead the pack for antioxidants!



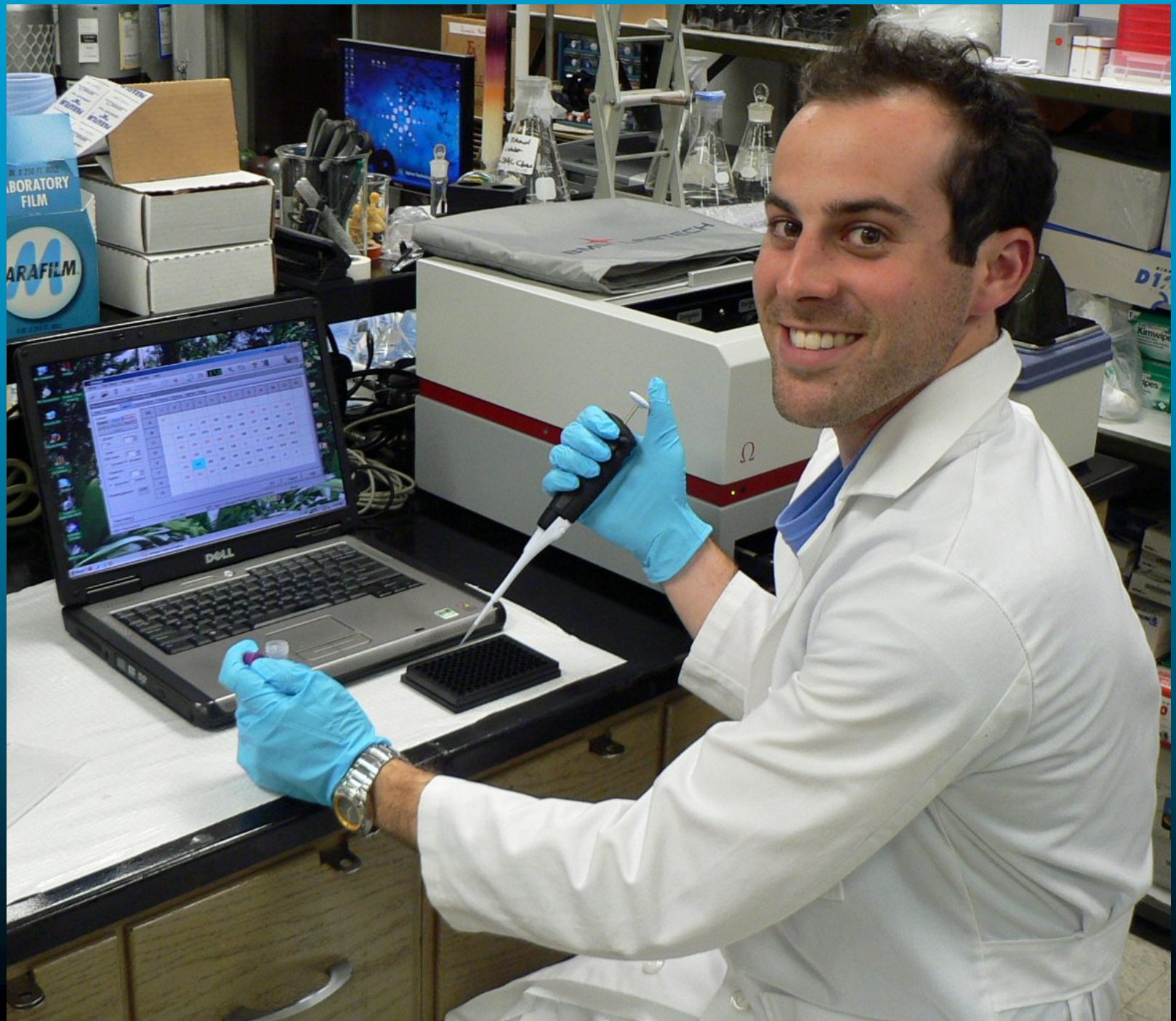
TOTAL ANTIOXIDANTS

(micromoles TE per serving)



www.georgiapekansfit.org

Source: Ronald L. Prior, Ph.D., J. Agric. Food Chem. 2004, 52, 4026-4037



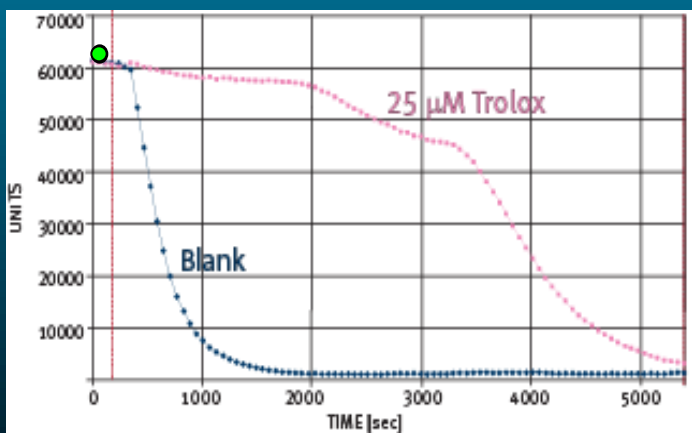
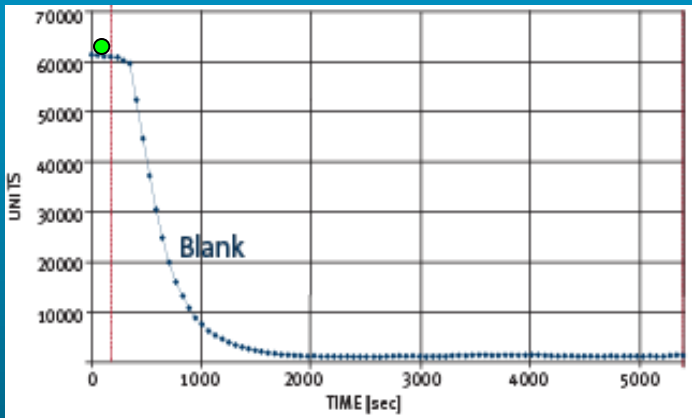
ORAC_{FL} Assay Principles



1) AAPH + Heat (37 C) \implies Free Radicals

2) Fluorescein + Free Radicals \implies Loss of fluorescence

3) Fluorescein + Free Radicals + Trolox \implies Maintain fluorescence longer



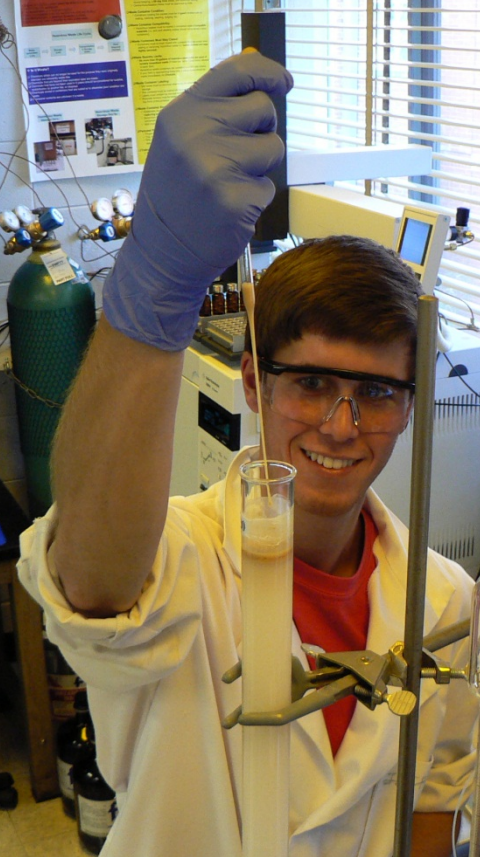
AAPH = 2,2'-Azobis(2-amidinopropane) dihydrochloride
Trolox = 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
(a water-soluble vitamin E analog)

Oxygen Radical Absorbance Capacity, Total Phenolics Content, and Total Procyanidins Content of Pecan Samples

Pecan cultivars*	H-ORAC _{FL} [§]	TPC [§]	TPrC [§]
Desirable	19,201	1,586	542
Kiowa	15,052	1,272	403
Elliott	18,647	1,300	479
Pawnee	17,513	1,282	407
Schley	17,107	1,236	414
Stuart	23,668	1,597	587
Sumner	19,686	1,410	465
Western Schley	17,790	1,398	520

* All pecan cultivars were defatted and then an 80% (w/w) acetonetic extract was prepared.

[§]H-ORAC_{FL} is in $\mu\text{mol Trolox eq./100 g nuts}$; TPC is in $\text{mg catechin eq./100 g nutmeat}$; and the TPrC assay for total procyanidins is in $\text{mg procyanidin B2 eq./100 g nutmeat}$.



Pecan Nutmeat

Soxhlet / Hexanes

Defatted Nutmeat

70:29.5:0.5% (v/v/v) AWAc

Crude Pecan Extract

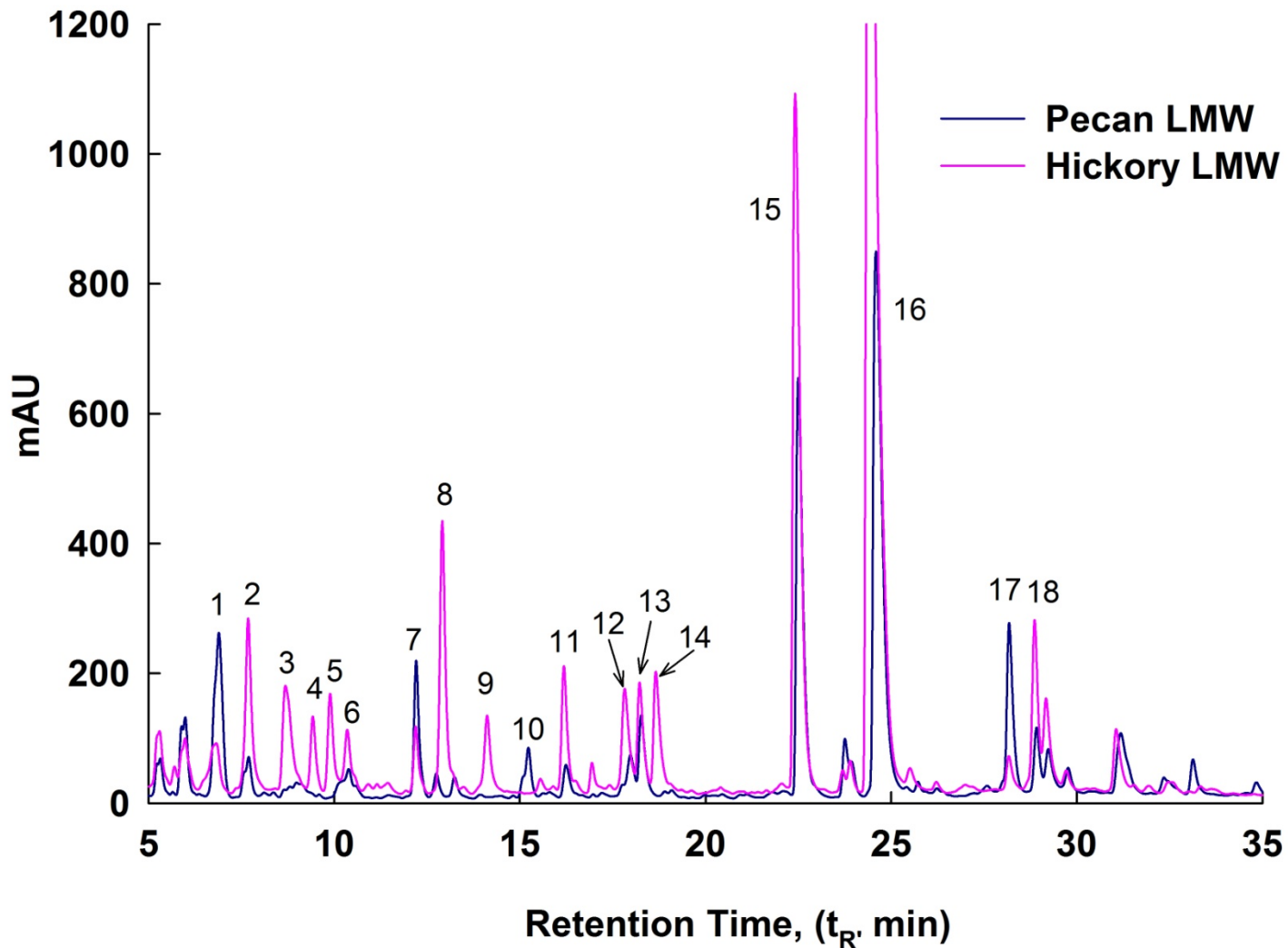
Yield
18.6±0.9%

**Lipophilic Sephadex LH-20 Column
95% (v/v) EtOH / 50% (v/v) Acetone**

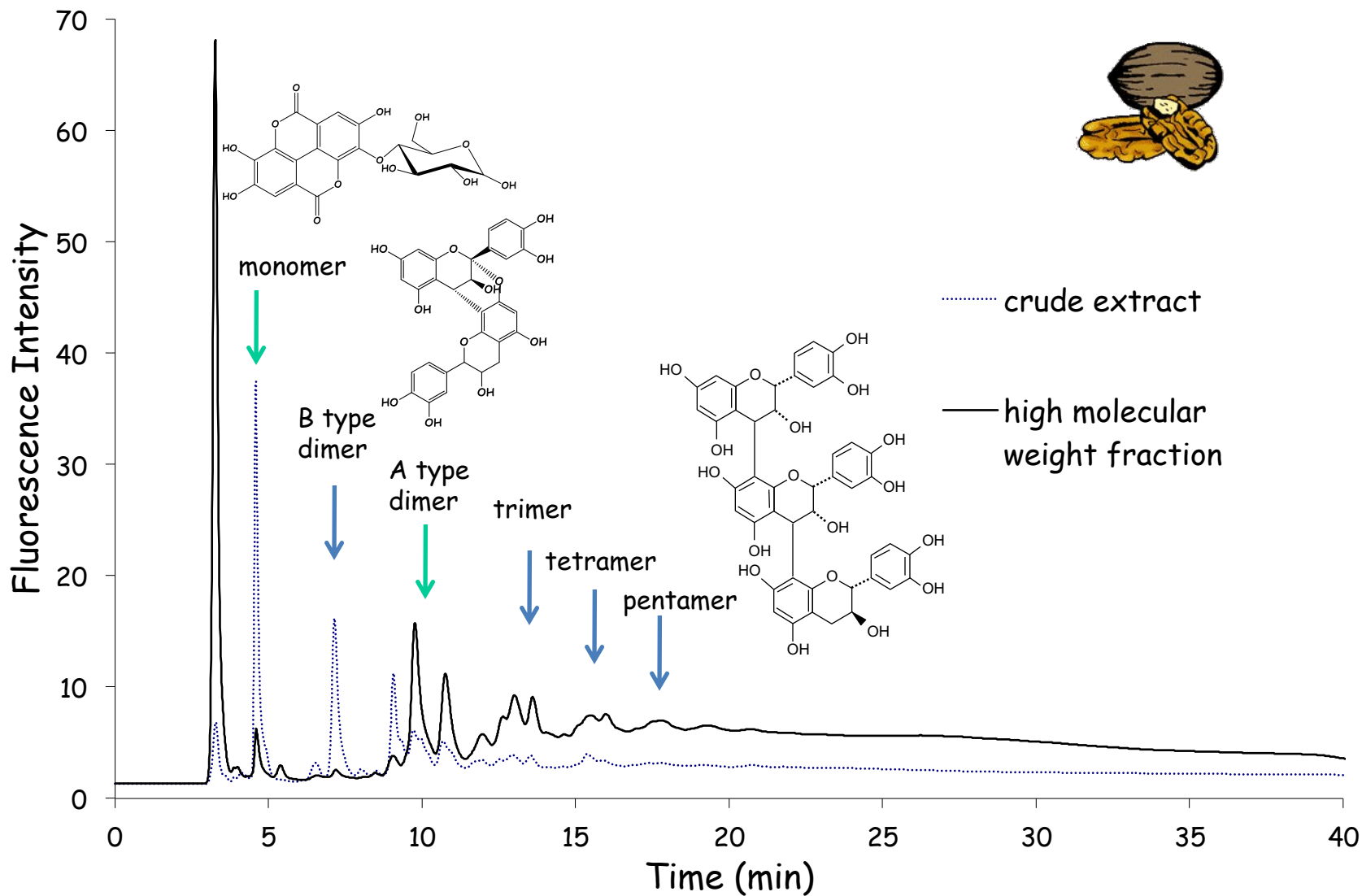
4 Low-Molecular-Weight Fractions (LMW)

Tannin Fraction

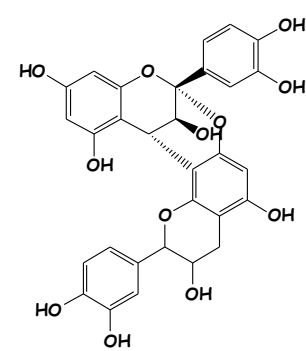
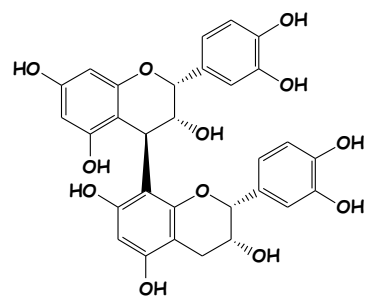
Comparison of Phenolic Fractions in Pecans and Chinese Hickory Nuts



Separation of Pecan PACs



Proanthocyanidin (PAC) Distribution in Pecans



Degrees of
Polymerization (DP)

Content within each DP
(mg/g fraction)^a

% Distribution of the
PACs

1

0.3

0.4

2

47.3

56.7

3

21.1

25.3

4 thru 6

14.8

17.7

> 6

tr

0

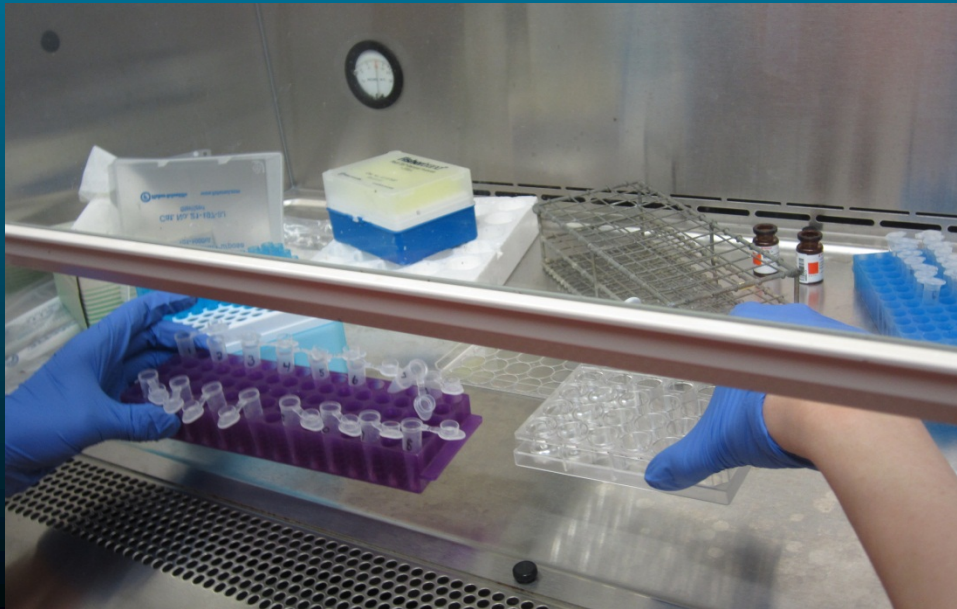
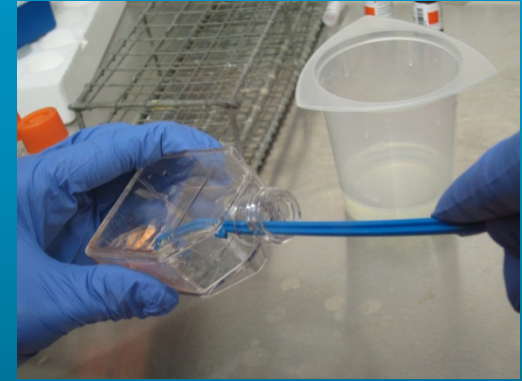
Total

83.5

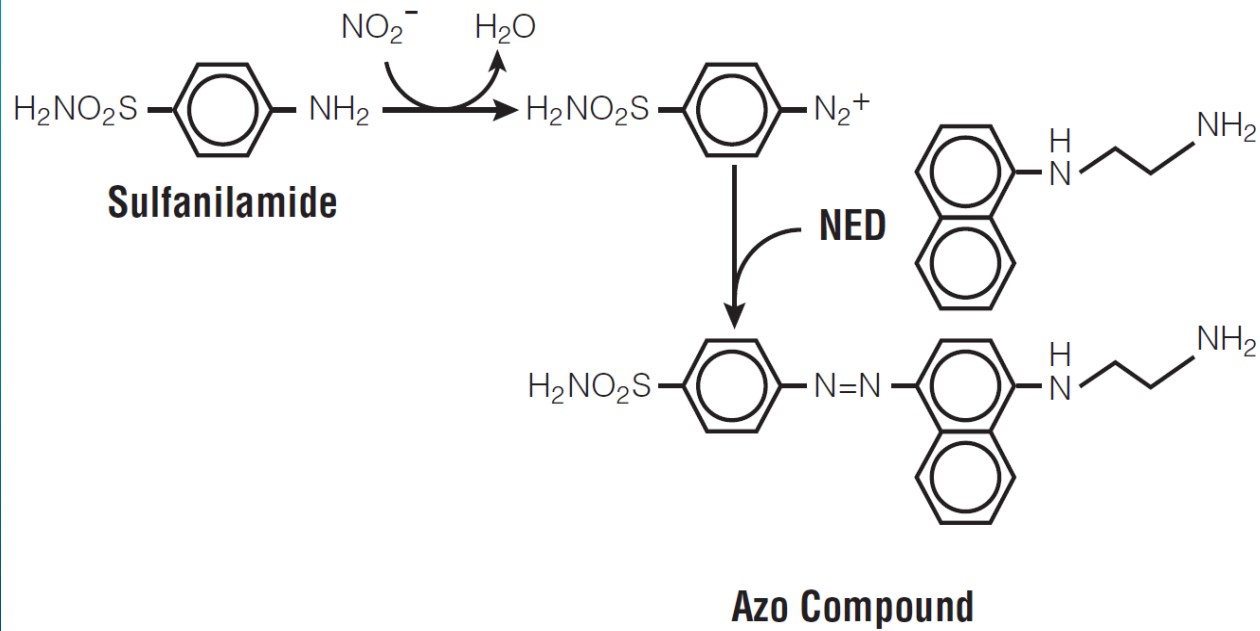
^aCharacterized by diol-phase HPLC with quantification completed using commercial standards with varying degrees of polymerization (Robbins, Ma, Wells, Greenspan & Pegg. 2014. *J. Agric. Food Chem.* 62:4332-4341).

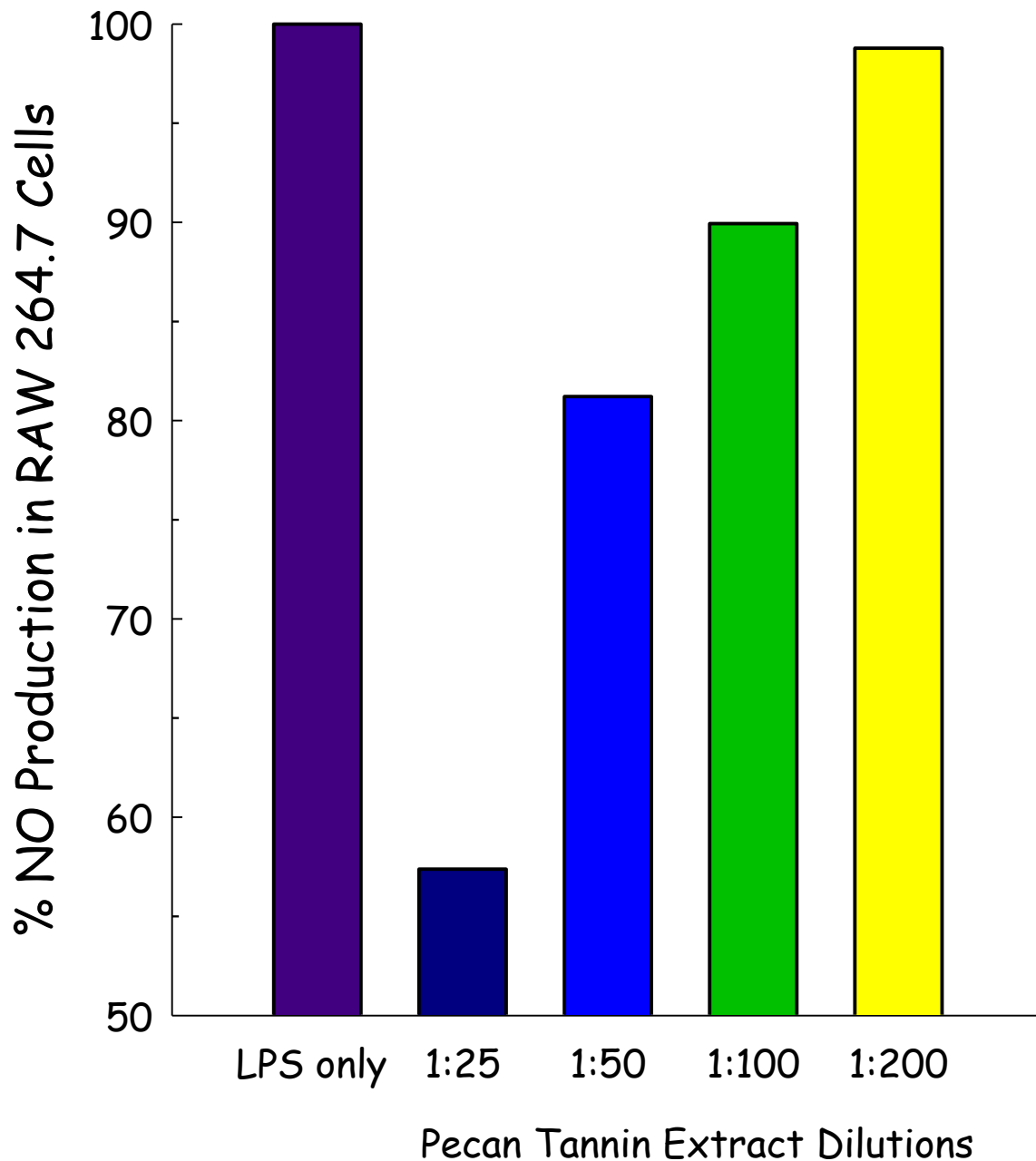


RAW 264.7 Macrophage Cell Lines



Measuring an Inflammation Index





0.5 mL of 1 μ g/mL
Escherichia coli
O11:B4 lipopoly-
saccharide (LPS) -
an irritant to initiate
the inflammation
process

Nutraceuticals & Functional Foods

Antioxidant Activity of Pecan Phenolics in a Cell-Based Assay

066-023

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Antioxidant Activity of Pecan Phenolics in a Cell-Based Assay

ABSTRACT

A significant contributing factor in the development of chronic illnesses, such as cancer and cardiovascular disease, is the consumption of antioxidants. Pecans, which are rich in antioxidants, have been shown to reduce risk of these diseases. The USDA ranks pecans among the top foods for antioxidant capacity due to their high levels of antioxidants. However, common chemical methods of measuring antioxidant capacity, such as ORAC, FRAP, and ABAP, do not accurately reflect the biological picture. In order to be more readily translated to in vivo situations, a cell-based assay was developed. Antioxidant phenolic extracts of defatted pecans, as well as standards, were applied to HepG2 cells and later Caco-2 cells. These pecan phenolics reduced the fluorescence of 2',7'-dichlorofluorescein (DCF) in HepG2 cells. In Caco-2 cells, 0.10 mg pecan phenolics/mL reduced the fluorescence of DCF by 50%. HPLC-ESI-MS/MS analysis determined the majority of the phenolics in pecan phenolics are small enough to be absorbed by the cells. These results are significant as they provide an assay accounts for biological pH and temperature, and are more relevant to the human body's antioxidant defenses.

METHODS



The fluorescence of DCF is measured every 5 min (n=3). Data points are plotted, and the area under the curve is measured and compared for a given extract and a control (media, no AC).

PRINCIPLE OF CELL-BASED ASSAY

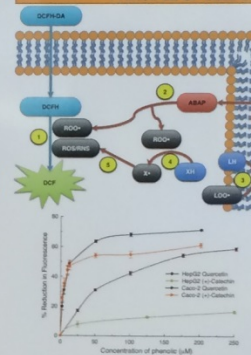


Figure 1: Antioxidant Performance of Quercetin and (+)-Catechin in HepG2 and Caco-2 Cells

Pathways for pecan antioxidant behavior in the cell

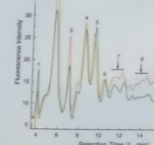
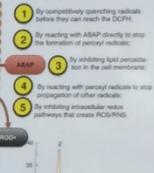


Figure 3: NP-HPLC Chromatograms for HMW Fractions from Raw and Roasted Pecans

RESULTS

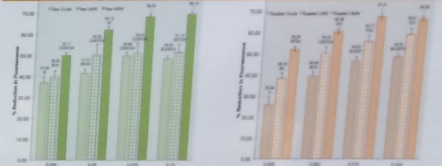


Figure 2(a): Cellular Antioxidant Activity of Phenolic Extracts from Raw Pecans

Figure 2(b): Cellular Antioxidant Activity of Phenolic Extracts from Roasted Pecans

Table 1: Characterization of PACs via HPLC-ESI-MS/MS

Peak No.	t_r (min)	DP	Unit Type	Charge	RT ² (min)	Flow	Summed Area
1	4.1	1	C	NA	289	2.81 ± 0.33	1.02 ± 0.10
2	5.6	2	EDC	B	577	12.63 ± 0.35	14.81 ± 0.30
3	7.1	2	EDC + EDCGC	B	593	3.77 ± 0.21	3.62 ± 0.71
4	8.6	3	EDC	B	865	7.02 ± 1.33	7.31 ± 1.04
5	9.4	3	EDC + EDCGC	B	881	5.43 ± 0.39	6.12 ± 0.72
6	10.5	4	EDC	B	1193	2.52 ± 0.18	2.25 ± 0.20
7	12.2	5	EDC	B	1461	0.96 ± 0.08	2.28 ± 0.13
8	14.5	5	EDC + EDCGC	B	1497	1.62 ± 0.21	2.30 ± 0.58

Abbreviations: DP: degree of polymerization; EC: epicatechin; C: catechin; EDC: epigallocatechin; GC: gallic acid.

DISCUSSION

- Caco-2 cells were used for pecan trials because experiments with (+)-catechin, a good standard for pecan phenolics, exhibited strong AOX activity.
- Caco-2 cells were used because they are derived from the GI system.
- Pecan phenolics exhibited strong AOX activity; HMW fractions were most effective.
- Reduced fluorescence by 69.1% (raw) and 67.3% (roasted).
- The PAC-rich HMW fractions were found to be ~80% dimers and trimers.
- Pecan phenolics were not cytotoxic, as seen in the MTT assay, as treatments and control wells showed no significant difference in absorbance ($p > 0.05$).

CONCLUSIONS

- Pecan phenolics exhibited strong antioxidant activity in Caco-2 cells, which are known to model the intestinal barrier; these results support the concept that phenolic monomers can be absorbed.
- The PAC dimers and trimers found in pecan phenolics appear to be small enough to be absorbed by cells and are therefore good dietary antioxidants – other foods, such as peanuts, have much larger PACs (DP>6). These have not been analyzed using CAA.
- Pecans show strong antioxidant activity by *in vitro*, *in vivo*, and cell-based assays; these results highlight potential unique healthful properties that make pecans one of the best dietary antioxidant sources.

2008-2014; Huthings et al., J. Nutr. 2011; 141, 58-62; Ma et al., J. Chromatogr. A, 2014, 1336, 84-81; Robbins et al., J. Agric. Food Chem. 2014, 62, 4332-4341; Wang & Joseph, Free Radic. Biol. Med. 1999, 27, 613-619; Wolfe & Liu, J. Agric. Food Chem. 2007, 55, 8998-8997.

There were 180 abstracts submitted to the NFF Division

Proven Nutritional Properties of Pecans

Good source of fiber

Trans-fat free

Cholesterol free

Sodium free

Protein (2.8 g)

Heart healthy



MUFA

Arginine

Phenolics

Vitamin E

3 Essential minerals

3 Essential water-soluble vitamins

Phytosterols

Packed with antioxidants!



Thank you