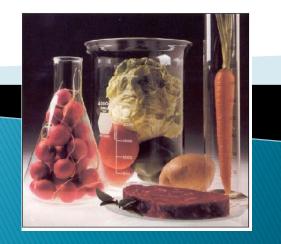
Novel Approaches for Food Verification & Authentication

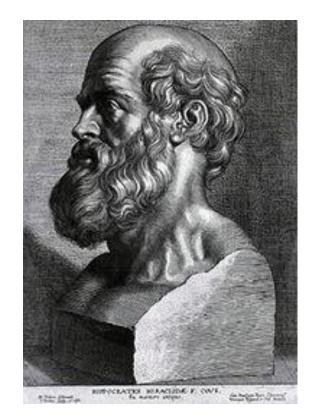
Alyson Mitchell, PhD John Kinsella Chair in Food, Nutrition and Health Department of Food Science & Technology, UC Davis Food Safety and Measurement Facility, UC Davis

AOAC 125th Regional Meeting Food Adulteration and Authentication UC Davis Robert Mondovi Institute Feb 19th 2014



Food is Complex

- "Let thy food be thy medicine."
 - Hippocrates (ca. 460 BC ca. 370 BC)
- But which foods?
 - Are all foods created equal?
 Are all apples created equal?
 Which one will provide me with what benefits?





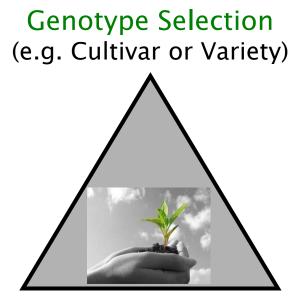
- Primary dietary source of vitamins, minerals, fiber and a wide array of non-essential nutrient phytochemicals in the American diet
 - polyphenolic antioxidants (e.g. flavonoids), carotenoids (e.g. lycopenes, β-carotene), alkaloids, glucosinolates, etc.)
 - Epidemiological studies indicate people who consume diets rich in F&Vs have a reduced risk of chronic diseases
 - stroke, type II diabetes, some cancers and heart disease
- These benefits are largely thought to be due to the synergistic activities of these bioactive phytochemicals
- Natural plant components in *traditional* medicine
- However, human studies on F&Vs are often contradictory and hard to interpret...
 - Why?

The Pharmacology Model Doesn't Apply:



- Non-essential nutrient bioactives represent a wide range of chemical structures with tremendous variability in foods
 - Little understanding of synergistic reactions between dietary components
 - Often comparing in vitro data with animal and human data
 - Types of intervention studies differ [acute, subacute, chronic]
 - Different endpoints and biomarkers are measured
 - Differences in dose and composition (foods)
- Limited understanding of chemical composition of foods
 - Cultivar variability, season, growing region, etc.,
 - Processing, storage, formulation, and packaging
- This lack of knowledge makes the medicinal or personalized nutritional use of foods difficult
- Manufacturing challenge
 - Ingredient sourcing and consistency in product composition

What Influences Non-Essential Nutrient Bioactives in Foods?



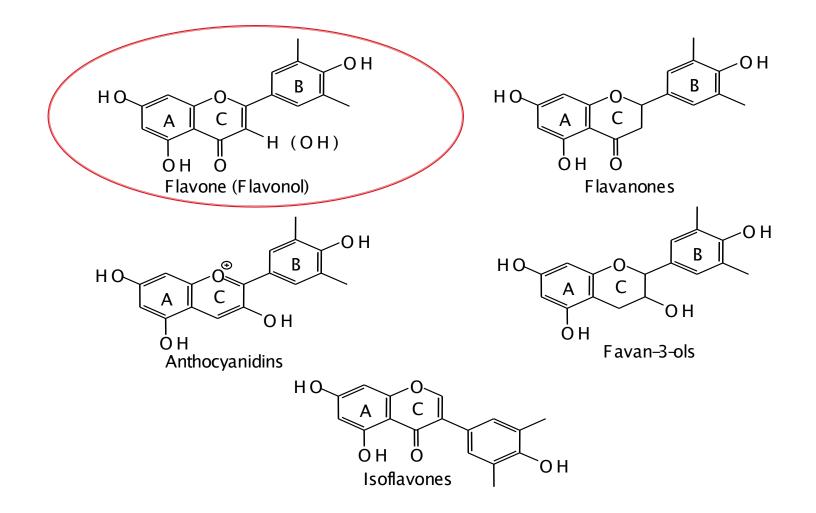
Agronomic and Environmental Pressures Secondary Plant Metabolites

Consumer

Post-Harvest Handling, Transport, Processing & Storage

Human Interface: Absorption, Bioavailability and Biological Activity

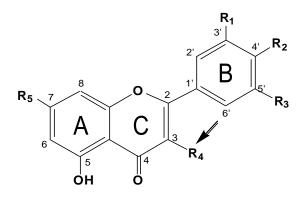
The Flavonoids



Roughly 2% of all hydrocarbons fixated in photosynthesis are converted into flavonoids and their derivatives

A Model Flavonol: Quercetin

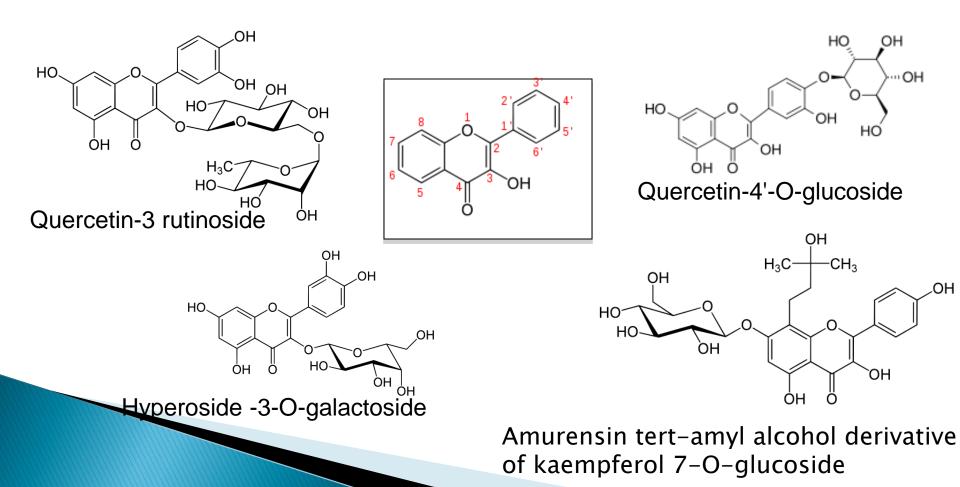
- Quercetin [3,3',4',5,7-pentahydroxyflavone]
- Hydroxylation, methylation and glycosylation pattern influences chemical diversity (5 forms)



Compound	R ₁	R_2	R_3	R ₄	R_5
Quercetin	OH	OH	Н	ОН	OH
Kaempferol	Н	OH	Н	OH	OH
Myricetin	OH	OH	OH	ОН	OH
Morin	OH	Н	OH	Н	OH
Isorhamnetin	OCH_3	OH	Н	ОН	OH

It Gets Even More Complex

- Flavonoids can also be acylated, malonylated and sulfated as well as methylated and glycosylated
- Glycosides can be mono-, di-, and tri-saccharide substituted
 - The 5 forms just became 20+ or more forms



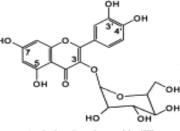
So Why Is this Important? The Source Matters

- The compliment of flavonoid conjugates differs in various F&Vs
- For example: The major US dietary sources of quercetin are
 - Onions: 210 mg/kg fresh weight
 - Apples: 30 mg/kg fresh weight
 - Most human and cell culture data is derived on forms not even found in foods



Quercetin-4'-0-6-D-glucoside (II)

Primary form found in onions *Highly Bioavailable*



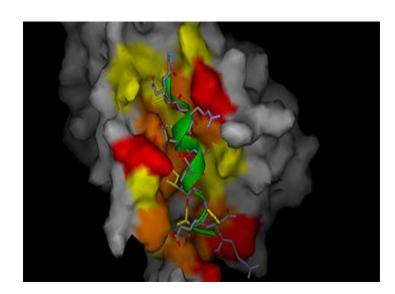
Quercetin-3-0-B-D-galactoside (III)

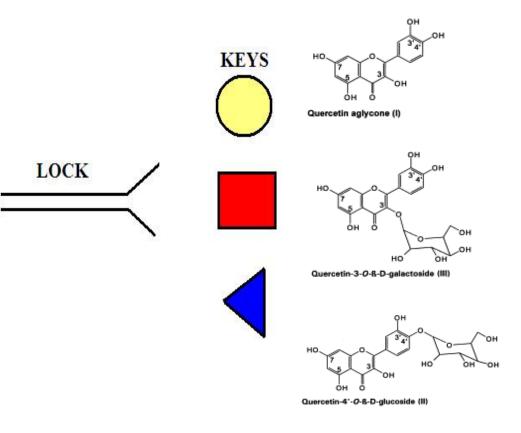
Primary form found in apples *Some Bioavailability*

Used in cell culture studies, clinical trials, sold as an ingredient *Poor Bioavailability*

Why Conjugation is Important

- Receptors recognize molecules largely by shape
- Protein interactions are specific, intermolecular distances are critical for interactions between amino acids and active sites on molecules





What Form is in These Products?



Characterizing Quercetin in Onions Variety Matters



variety	quercetin 3,4'-	quercetin 3-	quercetin 4'-	isorhamnetin	quercetin	sum	
,	diglucoside	glucoside	glucoside	4'-glucoside	aglycone		
Cougar	541.3 ± 3.9	42.0 ± 0.8	480.0 ± 4.2	$\textbf{79.2} \pm \textbf{0.4}$	256 <u>+</u> 2	1398.4 ± 3.3	
Don Victor	9.6 ± 0.4	Below LOQ	56.1 ± 1.2	$\textbf{7.2}\pm\textbf{0.2}$	20.1 ± 0.1	93.1 ± 1.7	
Gobi	94.6 ± 2.8	10.4 ± 0.2	102.5 ± 1.6	20.5 ± 0.6	29.8 ± 0.1	$\textbf{257.8} \pm \textbf{4.3}$	
Milestone	49.3 ± 0.7	53.8 ± 0.7	536.1 ± 3.3	16.7 ± 0.3	1047.0± 29.1	1703.0 ± 29.9	
Natasha	35.8 ± 2.8	4.3 ± 0.0	88.7 ± 2.2	11.6 ± 0.1	$\textbf{26.3}\pm\textbf{0.4}$	166.7 ± 4.8	
Warrior	242.4 ± 1.2	9.9 ± 0.0	$\textbf{229.9} \pm \textbf{2.2}$	34.1 ± 0.2	256.0 ± 2.4	739.1 ± 3.4	

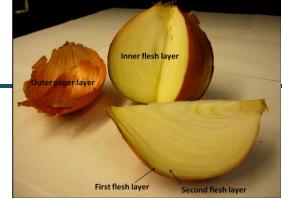
*Five primary forms found in onions LC-(ESI)MS/MS

Lee and Mitchell, 2011 J Agric Food Chem. 59(3):857-63

Q-TOF LC/MS Identification of Target Unkowns for Variety Verification

- The predominant factor influencing the compliment of flavonoids in food is genetics
 - Species and cultivar specific
 - This has important impact when sourcing ingredients that will have specific characteristics (e.g. bioactives, flavors, etc.,)
- Could Q-TOF LC/MS be used to identify varietal differences based upon a target unknown analysis of flavonoids?
- How would this data compare with non-target analysis of the same sample?
- Onions as a Model
 - Gills Onions, Oxnard CA

Flavonoid profiles in onions relatively simple



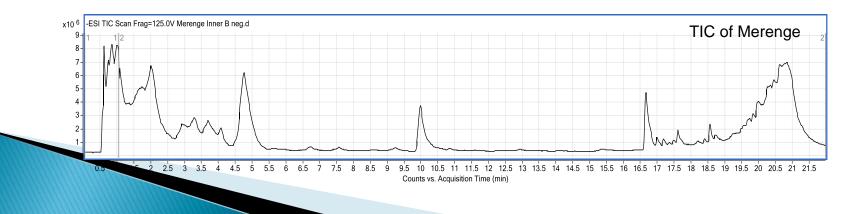
- Seven varieties of onions were evaluated
 - Yellow: Cowboy, Chief, Vaquero, Sommerset
 - Red: Red Rock, Salsa, Merenge
- Inner layer of the onion were separated from the outer layers and used in this study
 - Inner layers have limited anthocyanidins (red color)
 - Onions were lyophilized and extracted with 80% MEOH for 20 minutes
 - Anthocyanins will not be extracted under these conditions
 - All samples run in triplicate

Q-TOF LC/MS Analysis

- Methanolic extracts (80:20 MeOH:H₂0 v/v) were separated on a Poroshell 120 EC-C₁₈ (2.1 x 100 mm, 2.7 mm) column using a 1200 RRLC (Agilent Technologies)
- Mobile Phase:
 - A: 0.1% formic acid in H2O
 - B: 0.1% formic acid in ACN
 - 22 min gradient to 90% B at 0.4 mL/min
- Spectra were collected using a 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies)
- Data processed using Mass Profiler Plus (Agilent Technologies)

Building a Library

- A targeted unknown library was developed by importing the empirical formula of flavonoids from various databases into MassHunter PCDL Manager
 - Phenol-Explorer and ChemSpider
- We found 250 posible flavanoids to import
 - caution there are mistakes in these databases
- Narrowed down list into a library of 150 possible flavonoids in onions
- Searched against the TIC for these compounds



Phenol Explorer Library Polyphenol content of foods

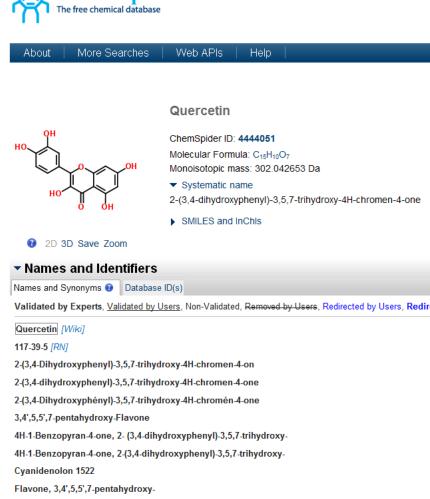
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2 Cyanidin	C15H11O6
3 Cyanidin3,5-O-diglucoside	C27H31O16
4 Cyanidin3-O-(6"-acetyl-galactoside)	C23H23O12
5 Cyanidin3-O-(6"-acetyl-glucoside)	C23H23O12
6 Cyanidin3-O-(6"-caffeoyl-glucoside)	C30H27O14
7 Cyanidin3-O-(6"-dioxalyl-glucoside)	C25H21O17
8 Cyanidin3-O-(6"-malonyl-3"-glucosyl-glucoside	
9 Cyanidin3-O-(6"-malonyl-glucoside)	C24H23O14
10 Cyanidin3-O-(6"-p-coumaroyl-glucoside)	C30H27O13
11 Cyanidin3-O-(6"-succinyl-glucoside)	C25H25O14
12 Cyanidin3-O-arabinoside	C20H19O10
13 Cyanidin3-O-galactoside	C21H21O11
14 Cyanidin3-O-glucoside	C21H21O11
15 Cyanidin3-O-glucosyl-rutinoside	C33H41O20
16 Cyanidin3-O-rutinoside	C27H31O15
17 Cyanidin3-O-sambubioside	C26H29O15
18 Cyanidin3-O-sambubiosyl5-O-glucoside	C32H39O20
19 Cyanidin3-O-sophoroside	C27H31O16
20 Cyanidin3-O-xyloside	C20H19O10
21 Cyanidin3-O-xylosyl-rutinoside	C32H39O19
22 Delphinidin3,5-O-diglucoside	C27H31O17
23 Delphinidin3-O-(6"-acetyl-galactoside)	C23H23O13
24 Delphinidin3-O-(6"-acetyl-glucoside)	C23H23O13
25 Delphinidin3-O-(6"-malonyl-glucoside)	C24H23O15
26 Delphinidin3-O-(6"-p-coumaroyl-glucoside)	C30H27O14
27 Delphinidin3-O-arabinoside	C20H19O11
28 Delphinidin3-O-feruloyl-glucoside	C31H29O15
29 Delphinidin3-O-galactoside	C21H21O12
30 Delphinidin3-O-glucoside	C21H21O12
31 Delphinidin3-O-glucosyl-glucoside	C27H31O17
32 Delphinidin3-O-rutinoside	C27H31O16
33 Delphinidin3-O-sambubioside	C26H29O16
34 Delphinidin3-O-xyloside	C20H19O11
35 Malvidin3,5-O-diglucoside	C29H35O17
36 Malvidin3-O-(6"-acetyl-galactoside)	C25H27O13
37 Malvidin3-O-(6"-acetyl-glucoside)	C25H27O13
 38 Malvidin3-O-(6"-caffeoyl-glucoside) 39 Malvidin3-O-(6"-p-coumaroyl-glucoside) 	C32H31O15
	C32H31O14
R ← ► H _ my_polyphenols _ 😒 _	

 Phenol Explorer Library (INRA)
 ~500 different polyphenols in over 400 foods

http://www.phenol-explorer.eu

- Provides molecular formulas of flavonoids
 - Numerous flavonoids have the same molecular weight due to variation in glycosylation patterns
- Onions
 - Isorhamnetin, Isorhamnetin 4'-glucoside, quercetin, quercetin 3,4'-diglucoside, quercetin 3-glucoside, quercetin 3-rutinoside, quercetin 4'-glucoside
 - Delphinidin 3-O-glucosyl-glucoside; <u>Cyanidin 3-</u> <u>O-(6"-malonyl-glucoside); Cyanidin 3-O-(6"-</u> <u>malonyl-3"-glucosyl-glucoside)</u>

ChemSpider



More...

- ChemSpider provides molecular formula, monoisotopic mass, systematic names and structure for a wide range of compounds
- All of these values can be downloaded into MassHunter PCDL for library development

MassHunter PCDL Manager

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	Neutral							
	Anion				Notes:			× ×

Single Search Results: 143 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Num Spectra
Kaempferol 3-O-(6"-malonyl-glucoside)	C24H22O14	534.10096			10.659				0
Kaempferol3-O-rutinoside	C27H30O15	594.15847			10.886				0
Dihydroquercetin	C15H12O7	304.05830			11.250		<u>458</u>	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydr	0
Quercetin3-O-glucoside	C21H20O12	464.09548			12.900		4444361	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-c	0
Quercetin4'-O-glucoside	C21H20O12	464.09548			16.640		<u>4478811</u>	2-hydroxy-4-(3,5,7-trihydroxy-4-oxo-4H-chromen-2	0
Kaempferol3-O-xylosyl-rutinoside	C32H38O19	726.20073			16.720				0
Quercetin3-O-rhamnoside	C21H20O11	448.10056			16.787		4444112	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-c	0
6,8-Dihydroxykaempferol	C15H12O8	320.05322			16.940				0
Isorhamnetin4'-O-glucoside	C22H22O12	478.11113			16.980				0
Quercetin	C15H10O7	302.04265			17.580		4444051	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chrom	0
Kaempferol	C15H10O6	286.04774			18.110		4444395	3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen	0
Isorhamnetin	C16H12O7	316.05830			18.183		<u>4444973</u>	3,5,7trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H	0

MassHunter Qualitative Analysis

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Background Spectra	+			3	Cpd 3: Dihydroquercetin	Dihydroquercetin	C15H1207	92.63	304.0576	304.0583	-2.27	
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🗉 📝 Cpd 4: Isorhamnetin	÷	V		7 Cpd 7: Quercetin3-0-glu	Quercetin3-0-glucoside	C21H20D12	57.14	464.0953	464.0955	-0.46
🗈 📝 Cpd 5: 6,8-Dihydroxykaempferol	÷	V					98.78	464.0962	464.0955	
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	×10.6		ide: -ESI EIC(463.0882) Sc	an Frag=125.0V Merenge Inner B	neg.d					
	1.8									
	1.0	1 1 2						16.634		
	1.6							16.634		
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m)	1.6 1.4 1.2	-						16.634		
🕾 Method Explorer: Find By Formular Onions with NO rete.	1.6 1.4 1.2	-						16.634		
- · · · · · · · · · · · · · · · · · · ·	1.6 1.4 1.2							16.634		
을 Method Explorer: Find ByFormular Onions with NO rete. 은 Chromatogram	1.6 1.4 1.2 _ × 1							16.634		
- · · · · · · · · · · · · · · · · · · ·	1.6 1.4 1.2 1 0.8 0.6							16.634		
Chromatogram	1.6 1.4 1.2 - × 1 0.8							16.634		
Chromatogram 9 Spectrum 9 General	1.6 1.4 1.2 1 0.8 0.6 0.4									
Chromatogram 9 Spectrum 9 General	1.6 1.4 1.2 1 0.8 0.6 0.4		3 35 4 45 5	55 6 65 7 7.5 8			. 14'5 15 16			95 20 205 2 ¹ 2
Gromatogram Spectrum General	1.6 1.4 1.2 1 0.8 0.6 0.4 0.2 0	05 1 15 2 25	3 3'5 4 4'5 5	55 6 65 7 75 8	8.5 9 9.5 10 10.5 11 11.5 12 Counts vs. Acquisition Time (1 14.5 15 15.5 16		25 18 185 19 19	9.5 20 20.5 21 2
Chromatogram Spectrum General File Open Actions	1.6 1.4 1.2 1 0 0 1 0 0 1 1 0 0 1 1 0 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1	0.5 1 1.5 2 2.5			Counts vs. Acquisition Time (1 145 15 155 16		18 185 19 19	95 20 205 21 2
Chromatogram Spectrum General Reports Find Compounds	1.6 1.4 1.2 1 0 0 1 0 0 1 1 0 0 1 1 0 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1	0.5 1 1.5 2 2.5		55 6 65 7 75 8 ▼ ① ₩ # Ω № %	Counts vs. Acquisition Time (1 145 15 155 16		7.5 18 185 19 19	95 20 205 21 2
Chromatogram Spectrum General Reports Find Compounds Find by Auto MS/MS	1.6 1.4 1.2 1 0 0 1 0 0 1 1 0 0 1 1 0 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1	0.5 i 1.5 2 2.5 Spectrum Results	▲ ● ● 1 ide: - Scan (16.585-16.884		Counts vs. Acquisition Time (1 14.5 15 15.5 16		/5 18 185 19 19	9 5 20 20.5 21 2
Chromatogram Spectrum General Reports Find Compounds Find by Auto MS /MS Find by Targeted MS /MS	1.6 1.4 1.2 08 0.6 0.4 0.2 0 0 0 0 0 0 0 0 0 0 0 0 0	0.5 i 1.5 2 2.5 Spectrum Results	<mark>ት</mark> ፈጋሮ 1	▲ Ⅲ ₩ № 品 (数)	Counts vs. Acquisition Time (i 14.5 15 15.5 16		7.5 18 18.5 19 18	is 20 20.5 21 2
Chromatogram Spectrum General Reopen Actions Reports Find Compounds Find by Auto MS/MS Find by Targeted MS/MS Find by Molecular Feature	1.6 1.4 1.2 08 0.6 0.4 0.2 0 0 0 0 0 0 0 0 0 0 0 0 0	0.5 1 1.5 2 2.5 Spectrum Results ↓ Q 😰 😻 ⊄ Cpd & Quercetin4'-0-glucos	▲ ● ● 1 ide: - Scan (16.585-16.884	▲ Ⅲ ₩ № 品 (数)	Counts vs. Acquisition Time (1 14.5 15 15.5 16		7.5 18 18.5 19 19	45 20 205 21 2
Chromatogram Spectrum General Reports Find Compounds ind by Auto MS/MS ind by Targeted MS/MS ind by Molecular Feature	1.6 1.4 1.2 1 0	Cpd 8: Quercetin4 ⁺ O-glucos	▲ ● ● 1 ide: - Scan (16.585-16.884	▲ Ⅲ ₩ № 品 (数)	Counts vs. Acquisition Time (1 145 15 155 16		1.5 18 18 5 19 19	\$5 20 20.5 21 2
Chromatogram Spectrum General General Reports Find Compounds Find by Auto MS/MS Find by Targeted MS/MS Find by Targeted MS/MS Find by Molecular Feature	= × 16 1.4 1.2 1 0.6 0.4 0.2 0.4 0.2 0.4 0.4 0.2 0.4 0.4 0.4 0.4 0.4 0.4 0.4 0.4	0.5 i 1.5 2 25 Spectrum Results ↓ Q 😰 🆋 🗸 1 Cpd & Quercetin4 ¹ -0-glucos	▲ ● ● 1 ide: - Scan (16.585-16.884	▲ Ⅲ ₩ № 品 (数)	Counts vs. Acquisition Time (1 145 15 155 18		75 18 185 19 19	<u>35 20 205 21 2</u>
	1.6 1.4 1.2 1 0	0.5 i 1.5 2 25 Spectrum Results ↓ Q 😰 🆋 🗸 1 Cpd & Quercetin4 ¹ -0-glucos	▲ ● ● 1 ide: - Scan (16.585-16.884	💌 🅕 ₩ 🔐 🗔 🦒 '	Counts vs. Acquisition Time (i 145 15 155 18		25 18 185 19 18	9.5 20 20.5 21 2
Chromatogram Chromatogram Spectrum General Reports Find Compounds ind by Auto MS/MS ind by Auto MS/MS ind by Autoeted MS/MS ind by Molecular Feature ind by MRM Find Compounds by Formula ind by Formula - Option:	= × 16 1.4 1.2 1 0.6 0.4 0.2 0.4 0.2 0.4 0.4 0.2 0.4 0.4 0.4 0.4 0.4 0.4 0.4 0.4	0.5 i 1.5 2 25 Spectrum Results ↓ Q 😰 🆋 🗸 1 Cpd & Quercetin4 ¹ -0-glucos	▲ ● ● 1 ide: - Scan (16.585-16.884	▲ Ⅲ ₩ № 品 (数)	Counts vs. Acquisition Time (i 145 15 155 16		7.5 18 18.5 19 18 	ds 20 20.5 21 2
Chromatogram Chromatogram Spectrum Conservation Chromatogram Spectrum Conservation Chromatogram Chromatog	= × 16 1.4 1.2 1 0.6 0.4 0.2 0.4 0.2 0.4 0.4 0.2 0.4 0.4 0.4 0.4 0.4 0.4 0.4 0.4	0.5 1 1.5 2 2.5 Spectrum Results	▲ ● ● 1 ide: - Scan (16.585-16.884	■ ① ₩ ₩ ₽ ₩ ₩ ₩ min) Meterge Inner B neg d	Counts vs. Acquisition Time (1972	457.1038		7.5 18 18.5 19 19	45 20 205 21 2
Chromatogram Chromatogram Chromatogram Chromatogram Consections C	= × 16 1.4 1.2 1 0.6 0.4 0.2 0 0 1 1 0.8 0.6 0.4 0.2 0 0 0 0 0 0 0 0 0 0 0 0 0	0.5 1 1.5 2 2.5 Spectrum Results	▲ ● ● 1 ide: - Scan (16.585-16.884	■ ① ₩ ₩ ₽ ₩ ₩ ₩ min) Meterge Inner B neg d	Counts vs. Acquisition Time (1972			7.5 18 185 19 19	45 20 205 21 2
Chromatogram Spectrum General General Reports Find Compounds Find by Auto MS/MS Find by Targeted MS/MS Find by Targeted MS/MS Find by Molecular Feature Find by MRM	= × 16 1.4 1.2 1 0.6 0.4 0.2 0 0 1 1 0.8 0.6 0.4 0.2 0 0 0 0 0 0 0 0 0 0 0 0 0	0.5 1 1.5 2 2.5 Spectrum Results	▲ ● ● 1 ide: - Scan (16.585-16.884	■ ① ₩ ₩ ₽ ₩ ₩ ₩ min) Meterge Inner B neg d	Counts vs. Acquisition Time (1972	467.1038 (M-H)-			<u><u>4</u>69</u>

 Compounds identified are evaluated based on exact mass, t_R and isotope spacing

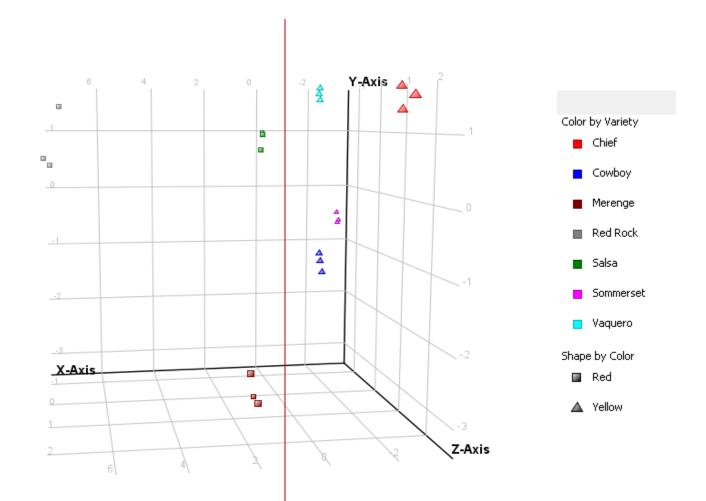
Data Mining Results

A compound list of 19 flavonoids were identified in the 7 varieties of onions:

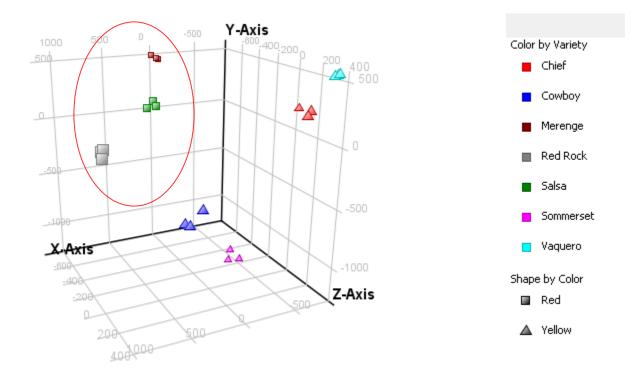
Delphinidin3-O-(6"-malonyl-glucoside) Dihydromyricetin3-O-rhamnoside Dihydroquercetin Isorhamnetin Isorhamnetin4'-O-glucoside Kaempferol Kaempferol 3-O-(6"-malonyl-glucoside) Kaempferol3,7-O-diglucoside Kaempferol3-O-acetyl-glucoside Kaempferol3-O-rutinoside Kaempferol3-O-xylosyl-rutinoside Quercetin Quercetin 3,7,4'-triglucoside Quercetin -O-diglucoside Quercetin3,4'-O-diglucoside Quercetin3-O-glucoside Quercetin3-O-rhamnoside Quercetin4'-O-glucoside



Principle Component Analysis on 19 Targeted Flavonoids for Varietal Difference



Principle Component Analysis On Non-Target Compounds Varietal & Color Difference



PCA analysis performed on data obtained using MFE

Non-pigment Compounds Responsible for Color Differentiation

Chief (yellow) vs. Merenge (red)

						Imperical							
Compound	Component 1 (80.91%)	[Red](raw)	[Yellow](raw)	Mass	Retention Time	Formula		Possible co	mpounds	(ChemSpi	der link)		Memo
401.2771@17.854668	2.448897	1	148,110	401.2771	17.854668	C17H35N7O4	<u>8128513</u>	<u>10137334</u>	<u>16569011</u>	<u>16572992</u>	<u>16575753</u>	<u>16575939</u>	
366.0947@12.518	2.449304	1	101,734	366.0947	12.518	C22H14N4S	<u>1730076</u>	<u>3422011</u>	4424188	<u>9489554</u>	<u>9958189</u>	<u>24762963</u>	(These are not all. There are 3 possible compounds more for this formula)
565.3451@18.173601	2.449187	1	66,407	565.3451	18.173601	C27H51N011	<u>19738984</u>						
471.1688@10.042999	2.44908	1	98,651	471.1688	10.042999	C28H25NO6	<u>2149487</u>	<u>2148324</u>	<u>2193205</u>	<u>2950892</u>	<u>4246898</u>	<u>3128669</u>	(These are not all. There are 94 possible compounds more for this formula)
678.2377@16.946	2.449251	1	72,799	678.2377	16.946	C30H38N4O14	<u>9149629</u>	<u>9160477</u>					
318.0735@16.160666	-2.4492757	137,619	1	318.0735	16.160666	C16H14O7	<u>89997</u>	2107568	<u>808939</u>	2854063	423348	<u>2793250</u>	(These are not all. There are 113 possible compounds more for this formula)
322.1623@15.643333	-2.4491868	159,363	1	322.1623	15.643333	C14H26O8	<u>81088</u>	469143	<u>469180</u>	<u>472191</u>	<u>487171</u>	<u>487181</u>	(These are not all. There are 31 possible compounds more for this formula)
304.0578@11.303	-2.4492521	279,442	1	304.0578	11.303	C15H12O7	<u>458</u>	<u>388626</u>	<u>19214</u>	<u>621332</u>	<u>46250</u>	5140861	(These are not all. There are 81 possible compounds more for this formula)
696.1538@11.1189995	-2.4489334	300,917	1	696.1538	11.1189995	C30H32O19	4478818	<u>8732006</u>	10188633	24843605	24844550	24844875	
552.1116@10.594334	-2.4492724	646,870	1	552.1116	10.594334	C24H24O15	<u>10476794</u>	13611569					

Compounds Tentatively Identified Responsible for Colored Differentiation

6,8-Dihydroxykaempferolkaempferol Dihydroquercetin Kaempferol 3-O-(6"-malonyl-glucoside) Kaempferol di glucoside Kaempferol di glucoside (isomer, different T_R) Kaempferol3-O-acetyl-glucoside Quercetin Quercetin3,4'-O-diglucoside Quercetin diglucoside (isomer, different T_R) Quercetin 3-rhamnoside

Conclusions

- A PCDL was established for 250 flavonoids
 - A PCDL was extracted from this specific for onion flavonoids and included 150 entries
- 19 flavonoids were identified in methanolic extracts of onions
 - Principle component analysis of these 19 target compounds demonstrate clear separation in varietal difference and color difference
- Non-target analysis resulted in similar results
 - Tentative identification of the top 10 flavonoids correlating strongly with color and variety differences
- More sampling of varieties grown under different conditions over time are needed to establish clear correlations

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 - Steven Fischer, PhD
- Gills Onions





