Clean Label Conference

May 24-25, 2022. Itasca, Illinois

The State of Natural Colorants: Advice on Applications to Updates on Recent Research

Slightly Redacted Version

M. Monica Giusti, Ph.D.

The Ohio State University

Dr. M. Mónica Giusti

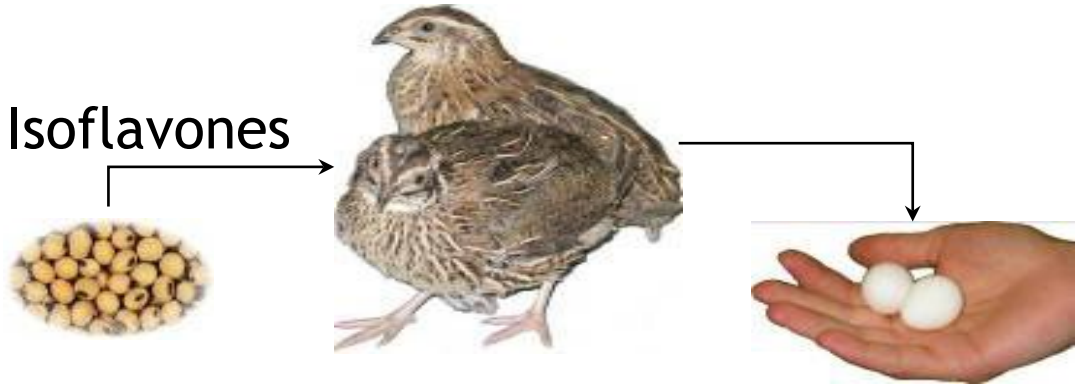
- Food Engineer, UNALM, Peru
- MS and PhD in Food Science, Oregon State Univ.
- Distinguished Professor, The Ohio State Univ., Food Science & Technology Dpt., CFAES

- >120 peer reviewed manuscripts, 25 chapters, 4 books
- Innovation and Teaching awards, OSU, IFT
- 8 patents granted, 3 more pending
- Fellow of the National Academy of inventors (since 2020)

THE OHIO STATE UNIVERSITY

Research Interest: Flavonoids

- **Isoflavones**
  - Phytoestrogens
- **Anthocyanins**
  - **Natural colorants**
  - Phytonutrients
- **Proanthocyanidins**
  - Urinary tract protection



Areas of work

- Analytical
- Horticultural
- Processing – food applications
- Bioavailability
- Health benefits


No Artificial Colors
Clean Label Ingredients
Colors Sourced from Plants

Consumer Trends

WANT TO AVOID…

- Synthetic ingredients
- Artificial colors
- Complex labels

WANT TO SEE…

- Natural
- Healthy
- Clean labels
- “super foods”

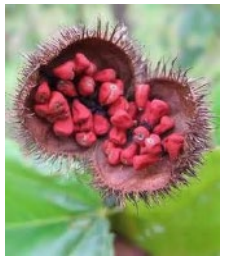
Why do we add colorants to food?

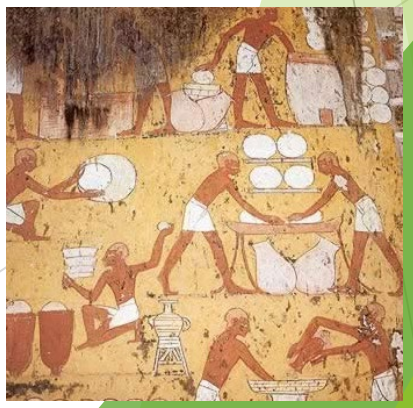
- < 85% of consumer buying decisions are potentially influenced by color
- Color has a major impact on flavor perception and flavor acceptance.
- Effective color usage drives consumer trial and acceptance.

Colors Added to Foods

Color has been added to foods since ancient times, and by cultures all over the world:

- (1500 BC) Egyptian wall paintings show color was used in candy
- (400 BC) Pliny the Elder spoke of artificial wine color
- Incas colored foods and fabrics with cochineal
- Mayans colored their food with annatto

Classes of Food Colorants in the USA

- Certified Colorants: Synthetic colorants
  - Chemically synthesized
  - EVERY batch must be FDA certified
- Colors Exempt from Certification
  - Colors from natural sources
  - Plant, animal or minerals pigments
  - OR… Nature identical

Food colorant uses

Only colorants determined to be safe by the FDA can be used (listed in 21CFR73)

- Enhance & correct colors already present
- Provide color identity to colorless foods
- Account for color loss during storage

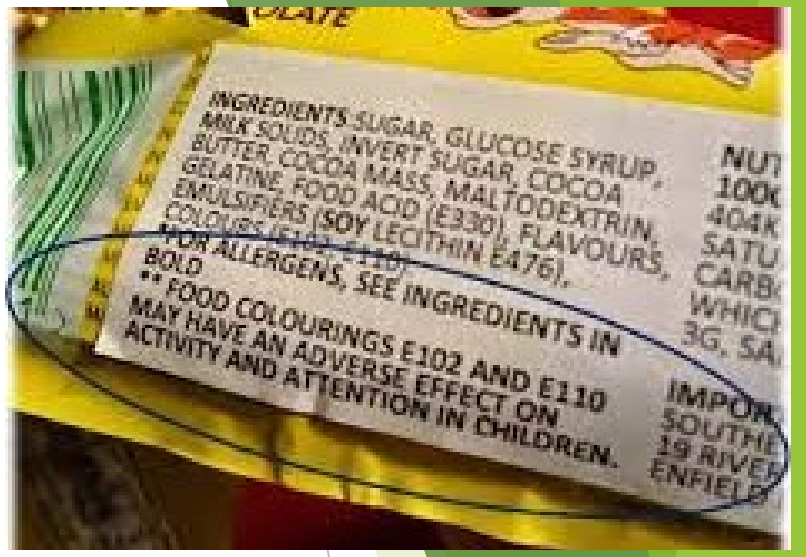
Food colorants should never be used to…

- Hide defects
- Deceive consumers

Synthetic Colorants Concerns

- Potential negative side effects
  - Allergies
  - Hypersensitivity
  - “The Southampton study”, UK – since 2007
    - Showed link between tested synthetic colorants and hyperactivity in children (ADHD)
- Regulatory changes in Europe, concerns all over the world.

European Food Safety Authority Warning Requirement



"consumption may have an adverse effect on activity and attention in children."

FD&C Red#40, Yellow#6, Blue#1

Beetroot red, Annatto, Paprika

NUTRI-GRAIN CEREAL BARS STRAWBERRY

Colors: Red No. 40, Yellow No. 6, Blue No.1

NUTRI-GRAIN SOFT BAKE BARS STRAWBERRY

Colors: Beetroot red, Annatto, Paprika extract

Warning in the USA?

- In 2011 FDA formed an expert panel to evaluate if warning labels were needed
- FDA decided not to require warnings, but recommended re-evaluation of the safety of all synthetic dyes.

Can we just remove synthetic colors?

- We eat with our eyes first…
- Consumers correlate color with
  - Identity of the product – product recognition
  - Flavor identification
  - Overall quality characteristics
  - Sometimes even safety and nutritional value!

Could we just use red to get red, and blue to get blue…???

- Replicating the colors from nature is not an easy task!

Where to start? Important considerations

- Your Product
  - What are the ingredients, processes, packaging
  - What color is the right color for your product?
- Who is your customer / target market
- Regulatory restrictions --- www.eCFR.gov

About the colorant choice

- Interaction with your Product
  - What color will it provide to your product?
  - How will it interact with other food components?
- Consumer perception
  - How will the color be listed?
  - Will it have a positive response from consumers?
- Regulations: Is the colorant allowed – at what level of usage?

Colors Exempt from Certification, 21CFR73

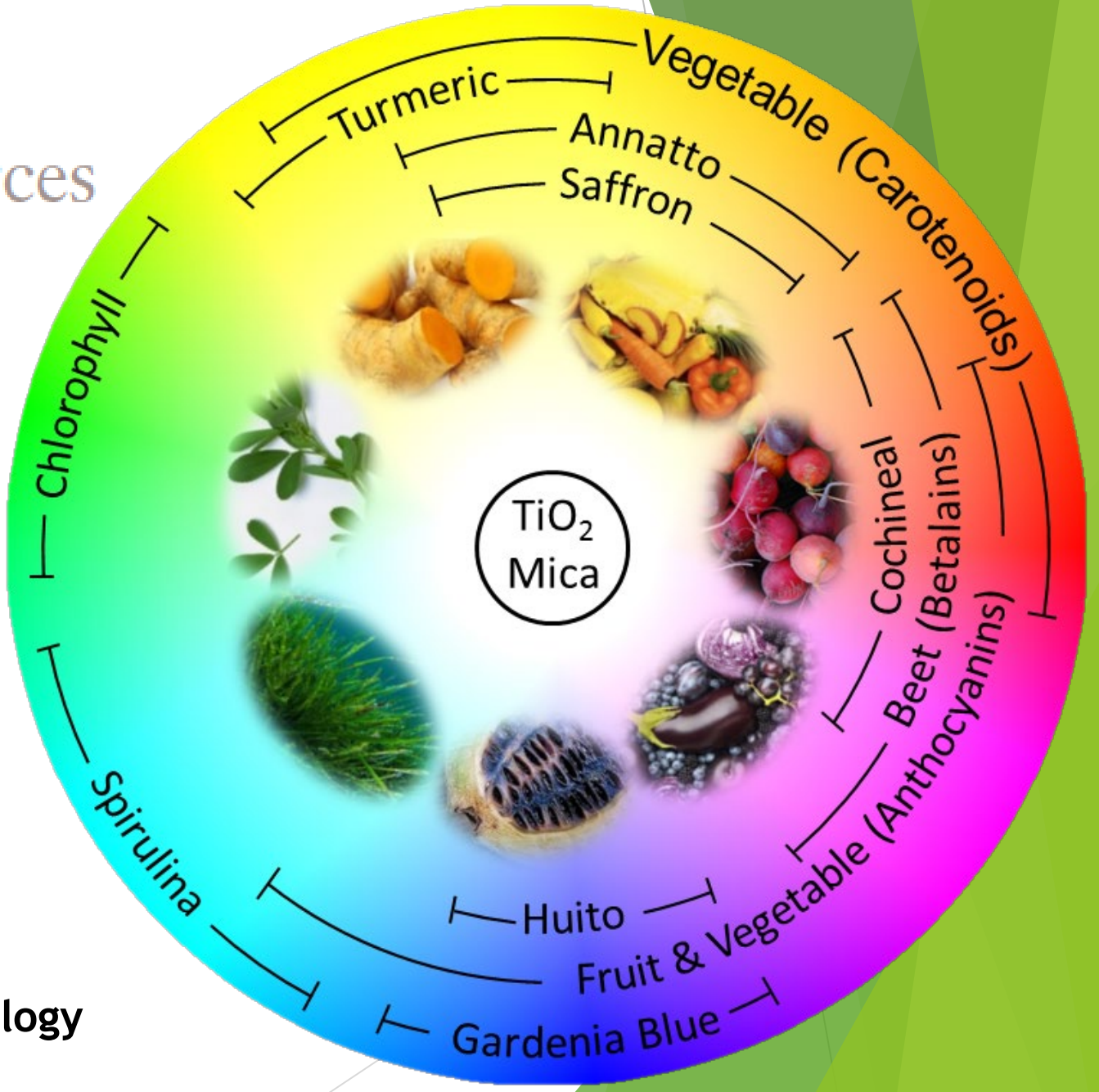
- Annatto extract
- Dehydrated beets (beet powder)
- [β]-Apo-8'-carotenal
- [β]-Carotene
- Butterfly pea flower extract
- Calcium Carbonate
- Canthaxanthin
- Caramel
- Carrot oil
- Cochineal extract; Carmine
- Cottonseed flour (toasted, partially defatted, cooked)
- Ferrous gluconate
- Ferrous lactate
- Fruit juice
- Grape color extract
- Grape skin extract (enocianina)
- Lycopene, tomato extract or concentrate
- Mica-based pearlescent pigments
- Paprika / Paprika oleoresin
- Riboflavin
- Saffron
- Soy leghemoglobin
- Sodium copper chlorophyllin
- Spirulina extract
- Synthetic iron oxide
- Titanium dioxide
- Turmeric
- Turmeric oleoresin
- Vegetable juice

A total of 39 are listed, with 30 for use in human food.

Natural Colorants: Food Colorants from Natural Sources

Gregory T. Sigurdson, Peipei Tang,
and M. Mónica Giusti

- Vegetable Sources
- Animal / Microbial Sources
- Mineral Sources



Annual Review of Food Science and Technology
Vol. 8:261-280 (2017)

Pigments in Plants: Nature is Colorful!!

Chemical Group	Pigments	Coloration	Occurrence (examples)
Tetrapyrroles	Chlorophylls	Blue–green	Broccoli, lettuce, spinach
Isoprenoid / Tetraprenoids	B-carotene	Yellow-orange	Carrots, melons, peaches
	Lycopene	Orange-red	Tomatoes, watermelon
Polyphenols	Anthocyanins	Orange-red-blue	Berries, red apple, red radish
	Flavonols	White–cream	Onions, coliflower
N-heterocyclic	Betalains	Purple/red-orange	Beets, cactus pear

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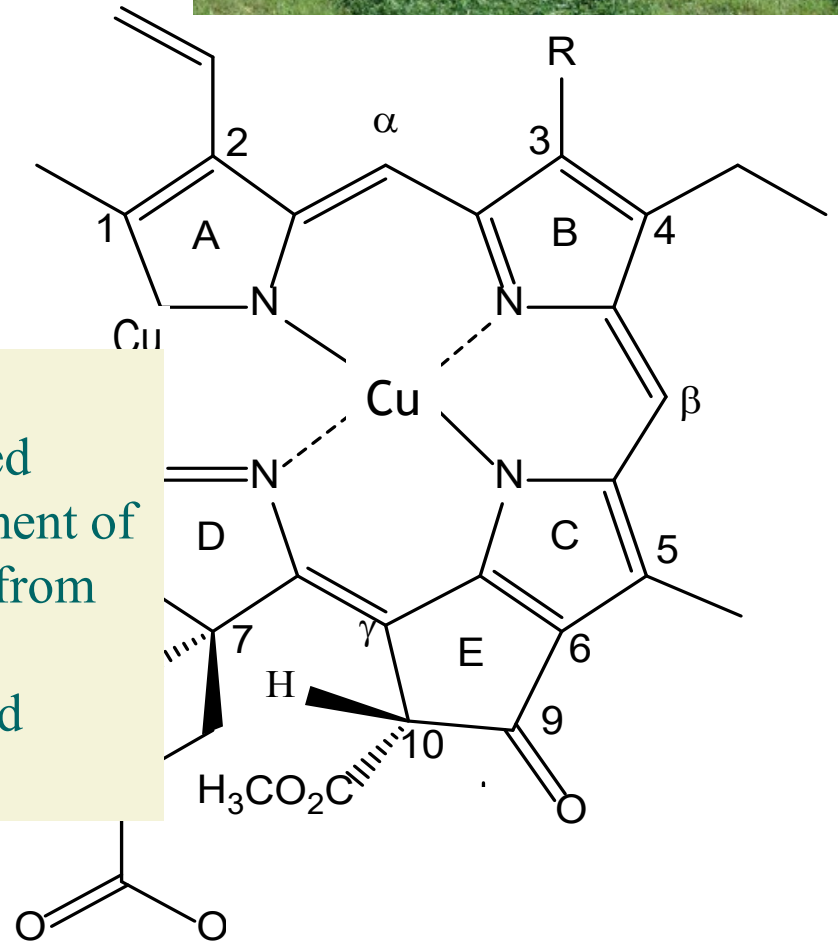
Only 1 colorant derived from Chrolophyll.

Chlorophyll-derived Colorant

Sodium copper chlorophyllin:

- Water soluble!!
- Mg^{2+} replaced with Cu^{2+}
- Restricted u
  dry mix cit

Identity. (1) The color additive sodium copper chlorophyllin is a green to black powder prepared from chlorophyll by saponification and replacement of magnesium by copper. Chlorophyll is extracted from alfalfa (*Medicago sativa*) using any one or a combination of the solvents acetone, ethanol, and hexane. (CFR 21 Part 73.125)



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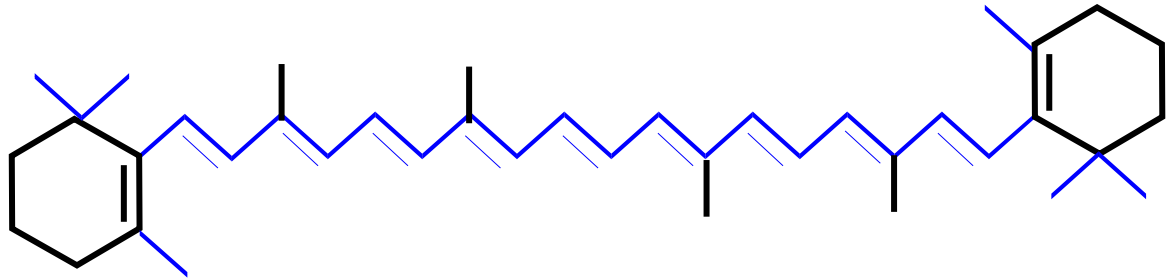
Several carotenoid-based or related colorants.

Carotenoids



- Colors range from yellow to orange to intense red
- Fat soluble
- Beta carotene: precursor to vitamin A

Commercial Forms of Carotenoids

- MANY!!!! Nature identical and from nature
- Physical properties
  - Liquid suspension in vegetable oil
  - Semi-solid suspension 25% in hydrogenated vegetable oil
  - Beadlet-water dispersible
  - Emulsion, beverage type

Annato

Canthaxanthin beadlets

β-carotene suspensions and beadlets

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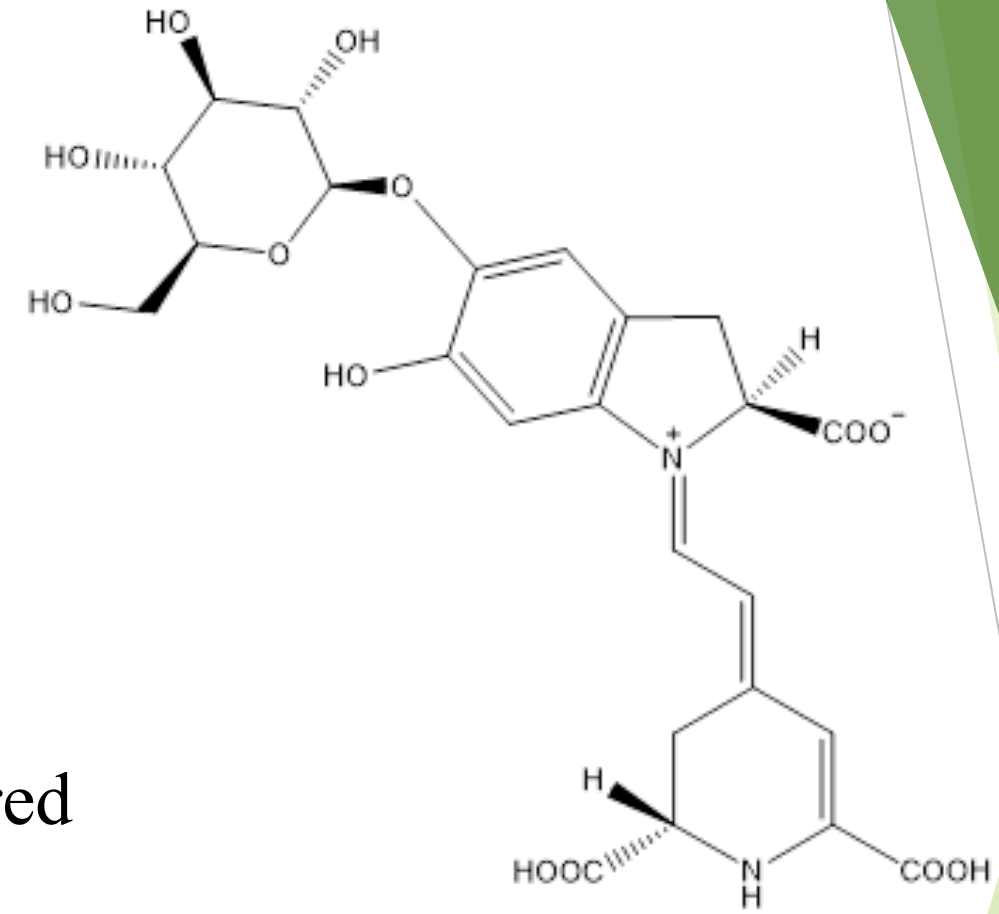
(a) *Identity.* (1) The color additive dehydrated beets is a dark red powder prepared by dehydrating sound, mature, good quality, edible beets. CFR 21, Part 73.40

Should be used according to GMPs.

Beet based colorant

Betalains



- From yellow to purple-red
- Water soluble
- Limited distribution in nature
- Not very susceptible to pH, works great at pH close to neutral
- Sensitive to light, heat, oxygen

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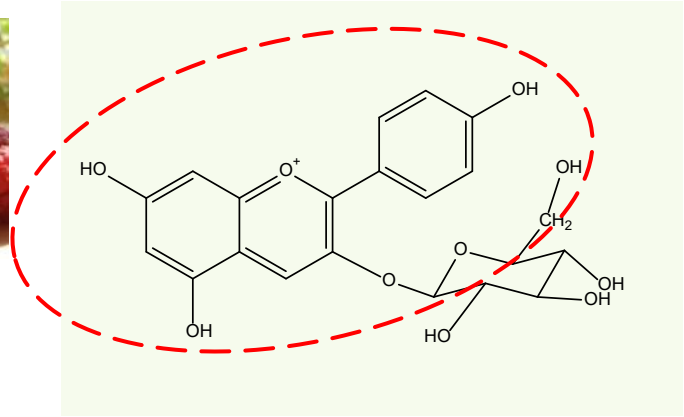
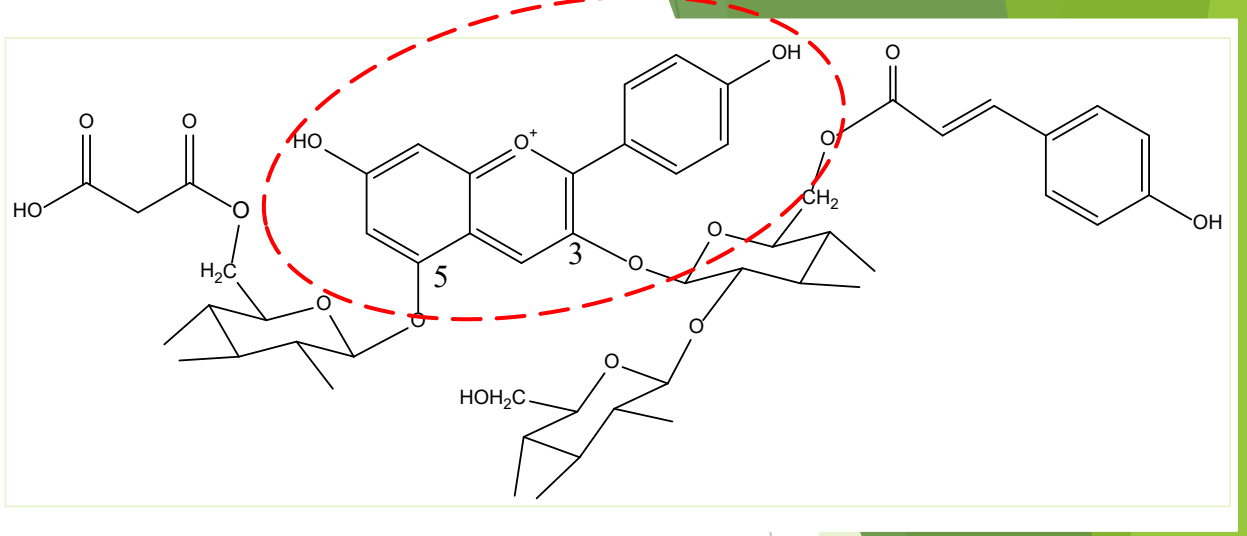
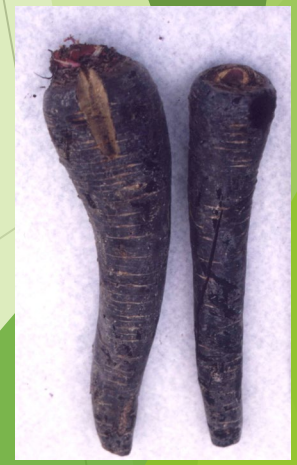
Anthocyanin-based Colorants.

Anthocyanin Sources







Berries & most fruits:
Simple pigments

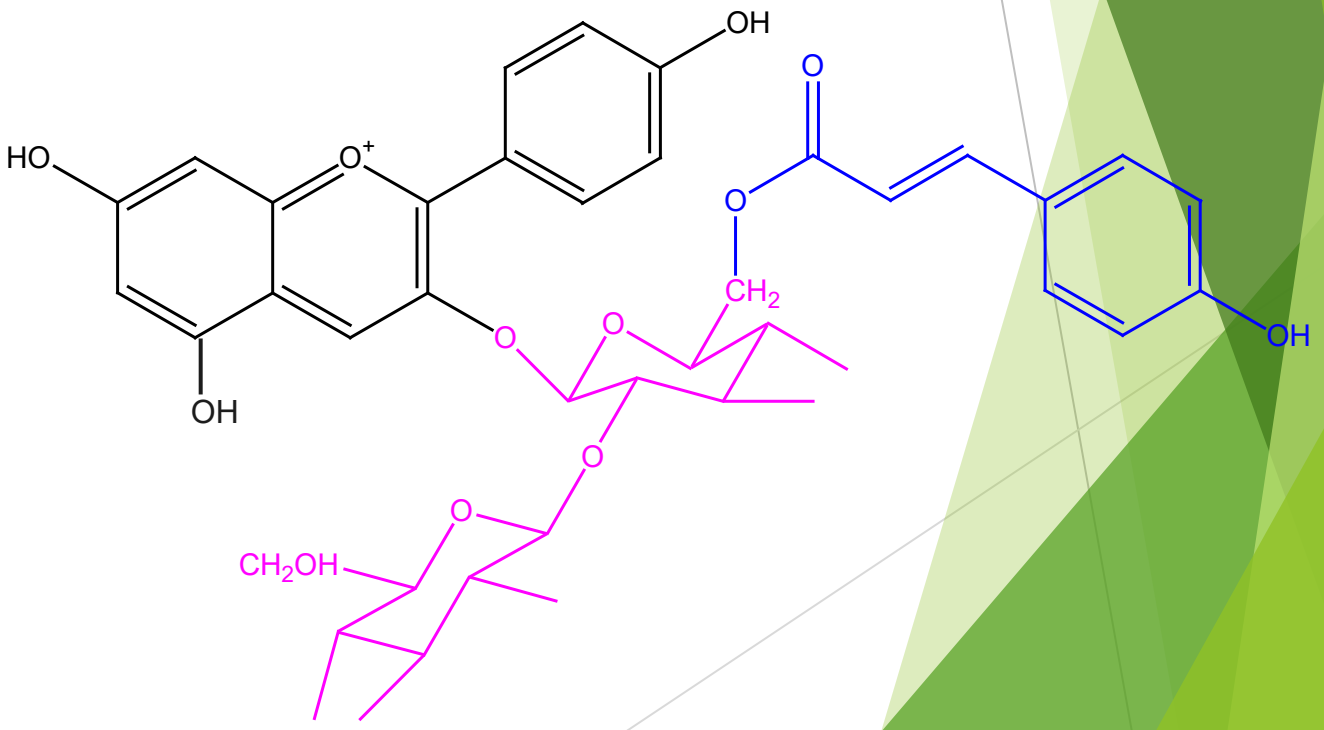
Other Sources:
Complex pigments

Fruit & Vegetable Juice Concentrates

- Pigments expressed and concentrated using:
  - Water as solvent
  - Physical means of extraction / concentration
  - Processes / aids already approved for juice manufacture
- Source must be edible
- NOT approved as juice
  - Alcohol / other solvents
  - Use of resins that separate based on chemical means / affinity

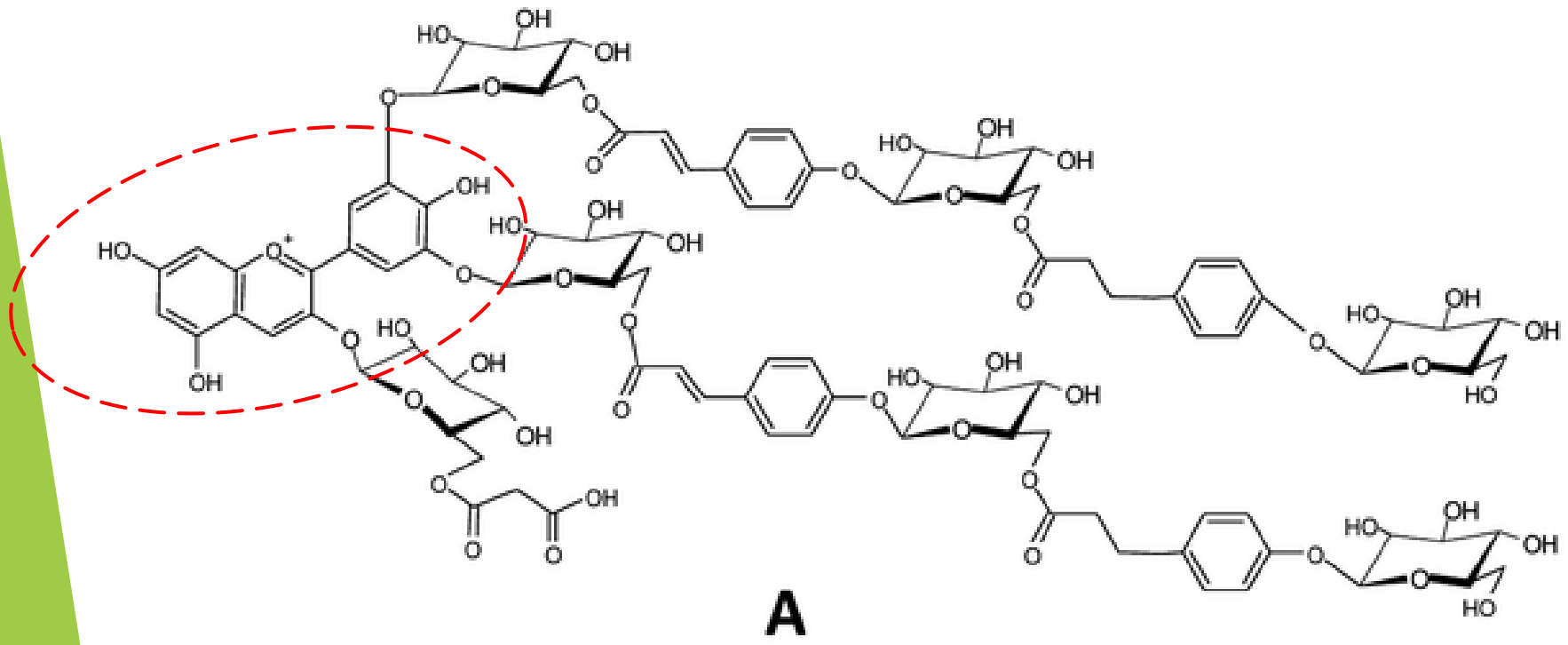
Anthocyanin colors are affected by…

- Chemical Structure
- Matrix composition
  - pH
  - Enzymes or pro-oxidants
  - Metals
  - Co-pigmentation
  - Bisulfite
- Other stressors
  - Temperature
  - Light
  - Oxygen



Just Approved, Oct 2021

- Butterfly Pea Flower Extract:
  - Anthocyanin-based colorant, produces blue colors even in low acid pH



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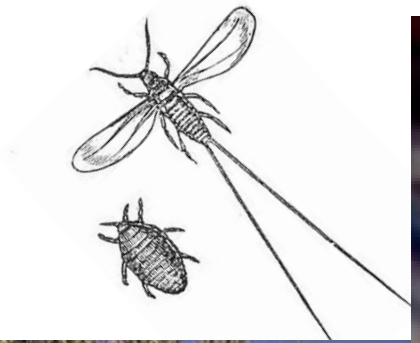
Other Interesting Colorants.

Caramel Colors

- Produced from heat treatment of sugars
  - Glucose, fructose, lactose, malt syrup, molasses, starch hydrolysates
  - Salts, acids or alkalis can produce a variety of colors.
- Many different applications:
  - Baking, desserts and confectionary
  - Sauces, soups and seasonings
  - Beverages
  - Snacks and cereals
  - Meats and poultry

Other natural sources of colorants

- **Turmeric**
  - From tubers of a plant ("*Curcuma longa*")
  - Yellow to orange
  - Curry pigments



- **Cochineal / carmine**
  - Source: dried insects
  - Colors form orange to brick red
  - Used in foods and many cosmetics!

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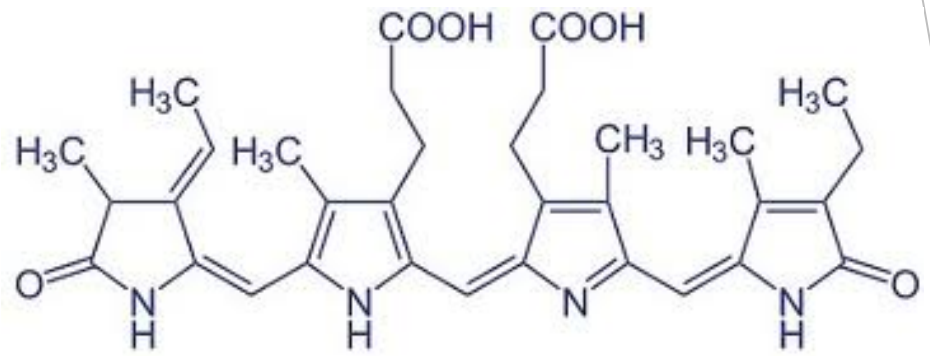
New Colorants Approved Since 2000

Recently Approved

- **Sodium Copper Chlorophyllin** (2002)
- **Tomato lycopene extract; tomato lycopene concentrate** (2005)
  - Red to dark brown oleoresin extracted with ethyl acetate from fresh, edible varieties of the tomato. The coloring is lycopene.
- **Mica-based pearlescent pigments** (2006)
  - Platelets of potassium aluminum silicate (mica) with titanium dioxide. Part transmittance, reflection and interference of light. Use in cereal, confectionary, spirits, alcohol.

Spirulina (2013)



- Green to blue in color
- Edible cyanobacterium, primarily from *Arthrospira platensis* and *Arthrospira maxima*.
- Phycocyanins and chlorophyl
- Main safety concern: production of toxic compounds by some cyanobacteria.

Spirulina Extract as Food Colorant

- GRAS Self affirmation, 2002 – not as colorant, but ingredient with color
- FDA approved Spirulina as Food Colorant for candy and chewing gum in September 2013
- Additional uses approved later, including frosting, dairy products, other desserts, gelatin, cereals, according to GMPs

Approved 2019

- **Soy leghemoglobin** (2019):
  - Product of controlled fermentation of a non-pathogenic and non-toxicogenic strain of the yeast, *Pichia pastoris*, genetically engineered to express soy leghemoglobin protein. It imparts a reddish-brown color.
  - Behaves like meat myoglobin

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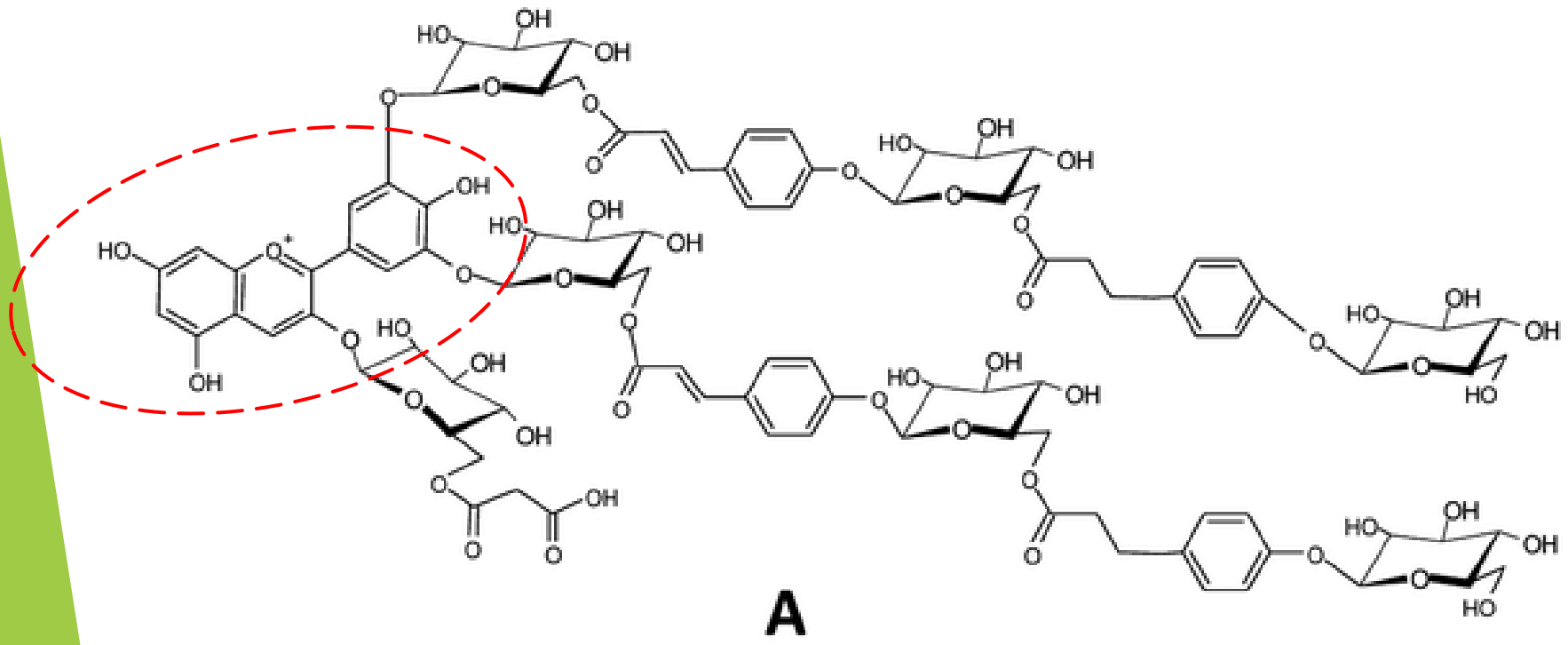
  - Anthocyanin-based colorant, produces blue colors even in low acid pH



Transitioning to Colorants from Natural Sources

Challenges:

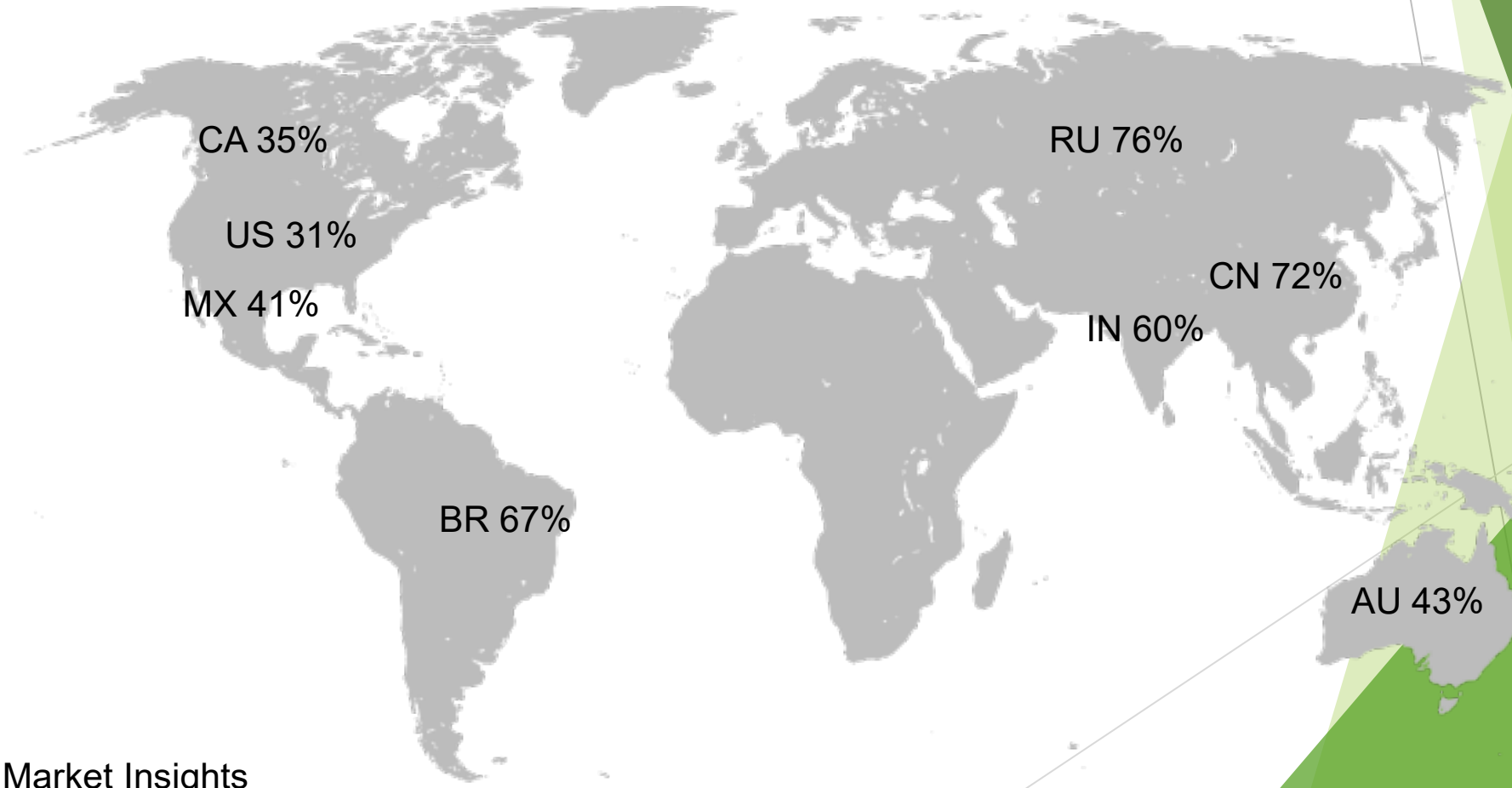
- **Finding the "Right" color**
- Compatibility with matrix
- Color and pigment stability
- Possible undesirable aromas / flavors
- Higher costs? Changes in the process?

Opportunities:

- Consumer perception / increased demand
- Standardizing formulations!!!
- Added value: potential health benefits?
- Coloring foodstuffs (i.e., natural plant extracts or concentrates) in place of coloring additives.

Trends Towards Color from Naturals Sources

- Proportion of consumers that report to be very/extremely concerned about food colorings



INNOVA Market Insights

Clean Label Conference

May 24-25, 2022. Itasca, Illinois

Recent Research on Anthocyanin-based Colorants

M. Monica Giusti, Ph.D.

The Giusti Phytochemicals Laboratory.

The Ohio State University

https://u.osu.edu/giustilab/

Stabilization and Color Enhancement of Anthocyanins

- The anthocyanin chemical structure
- Horticultural factors
- Copigmentation
- Metal complexation
- Anthocyanin-protein interactions
- Pyranoanthocyanins
- Microencapsulation

The Giusti Phytochemicals Laboratory. https://u.osu.edu/giustilab/

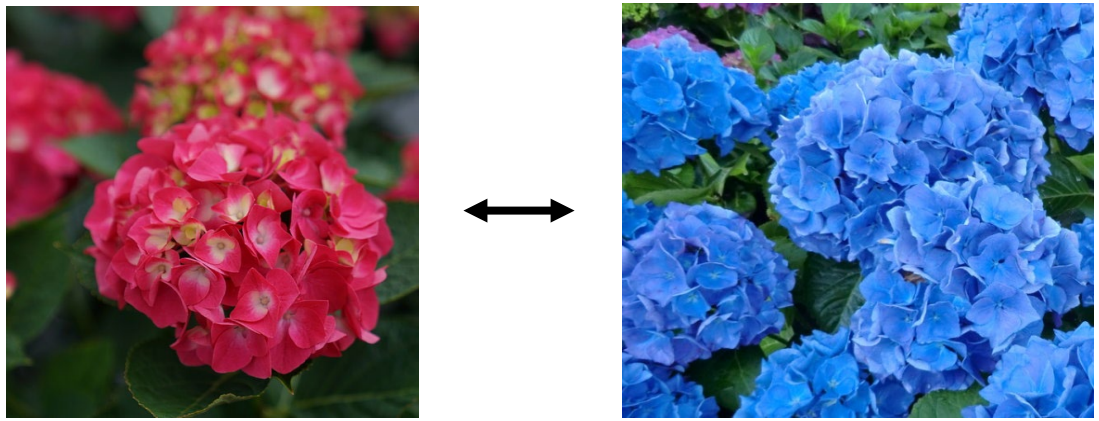
Horticultural Factors Affecting Phenolic Accumulation

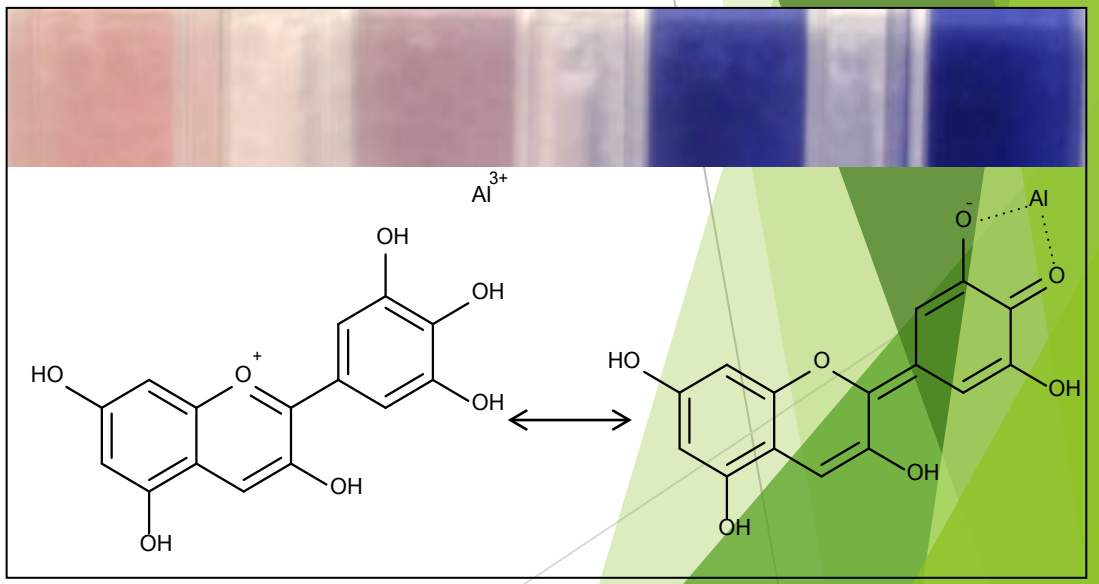
- Plant domestication can alter (reduce) anthocyanin and phenolic content
- Cultivar selection and growing conditions affect pigment concentration and composition
- Insect infestation on blueberry induced phenolic accumulation and altered anthocyanin profile

Ahmadiani, N; Robbins, R; Collins, T; **Giusti, MM.** 2014. Anthocyanins (…) of red cabbage *J. Agric.Food Chem.* 62: 7524–7531. Zhou, Y; **Giusti, MM**; Parker, J; Rodriguez-Saona, CR. 2016. BMSB Alters Blueberry… *Environ Ent*. 45 (5): 1227-1234.

Metal Chelation Affects Anthocyanin Color and Stability

- Evaluate the effect of anthocyanin structure on color expression of chelate
- Investigate stability of chelates

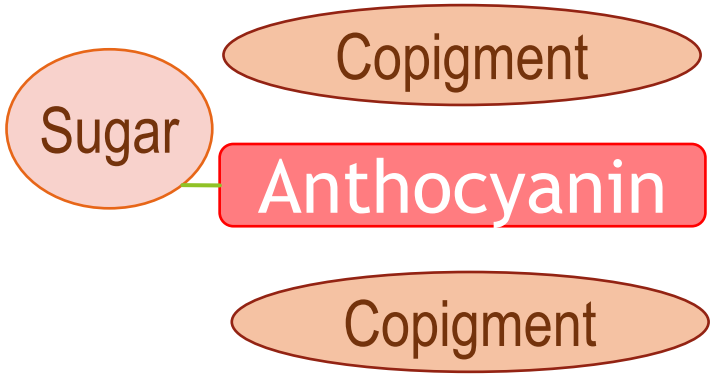


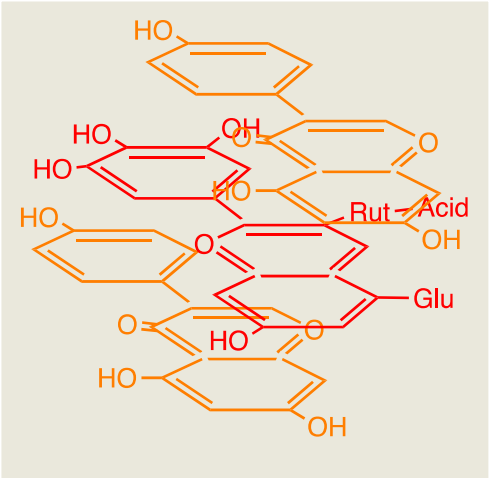


Sigurdson, GT; Robbins, RJ; Collins, TM; **Giusti, MM**. 2016. Metal Ions & Cyanidin. *Food Chem.* 208: 26-34. Sigurdson, GT; Robbins, RJ; Collins, TM; Giusti, MM. 2017. Spectra & Color of Metal Chelates of Acylated Cyanidin." Food Chem. 221: 1088-1095. Tang, P; Giusti, MM. 2020. Metal Chelates of Petunidin Derivatives Enhanced Color and Stability. Foods 9, 1426. https://doi.org/10.3390/foods9101426. .

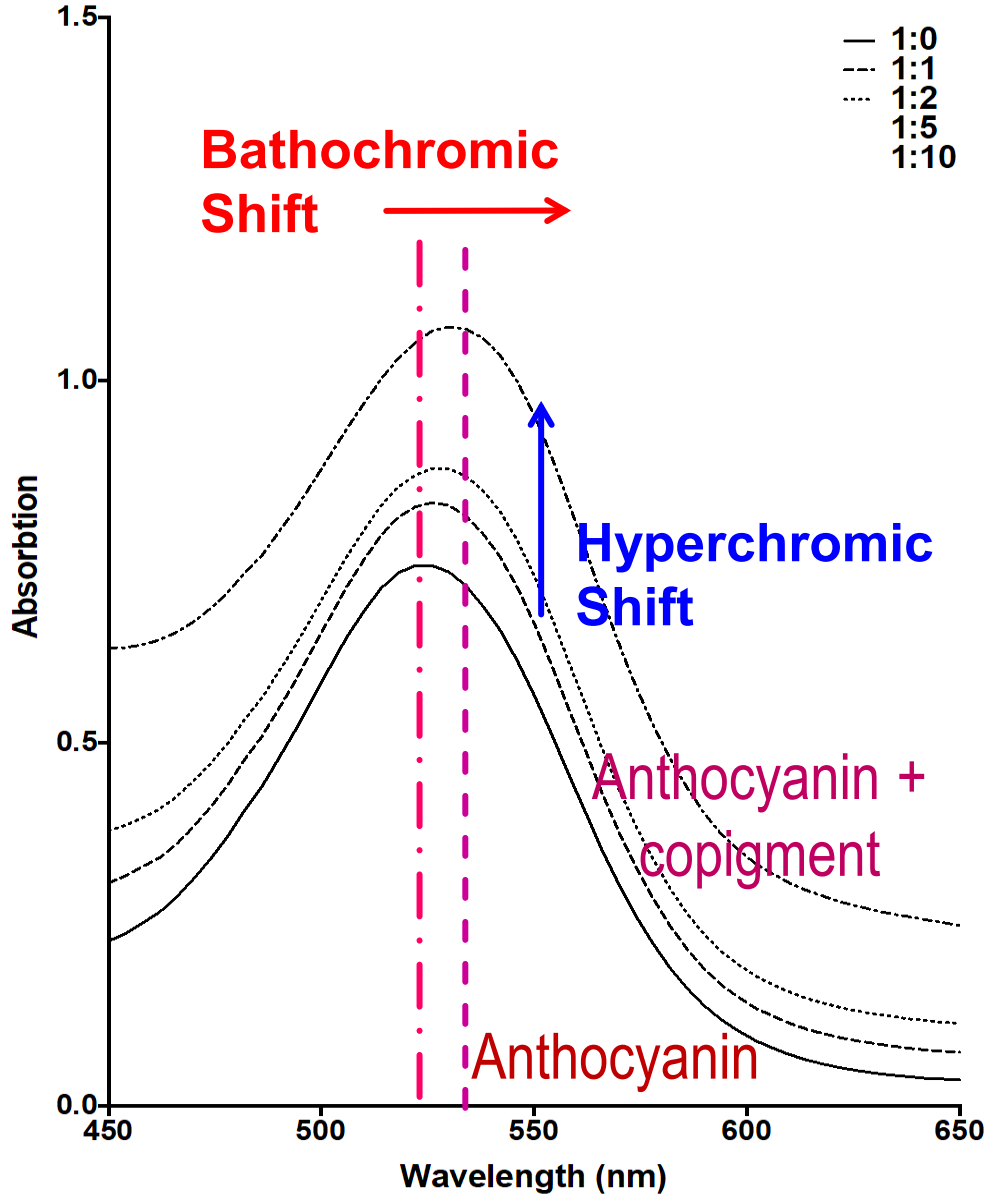
Anthocyanin Copigmentation

Anthocyanin color may be enhanced and stabilized by co-pigments



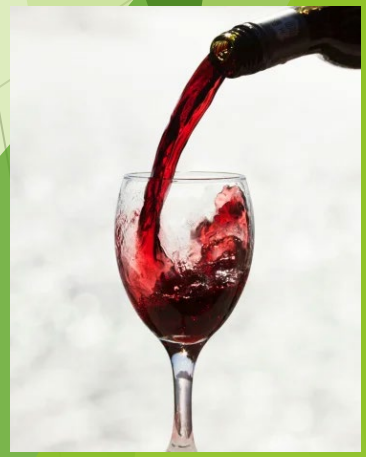


Intermolecular Copigmentation



Pangestu, NP; Miyagusuku-Cruzado, G; **Giusti, MM**. 2020. Copigmentation & Anthocyanin Stability. *Foods* 9 (10), 1476.. Ren, S; Jimenez-Flores, R; Giusti, MM. Interactions of anthocyanin and whey protein: A review. Comp.Rev Food Sci Food Safety. CRF3-2021-0442.R1. Gordillo, B; Sigurdson, GT; (…); Giusti, MM. (2018). Color and stability of naturally copigmented anthocyanin-grape colorants. Food Res Int, 106, 791-799.

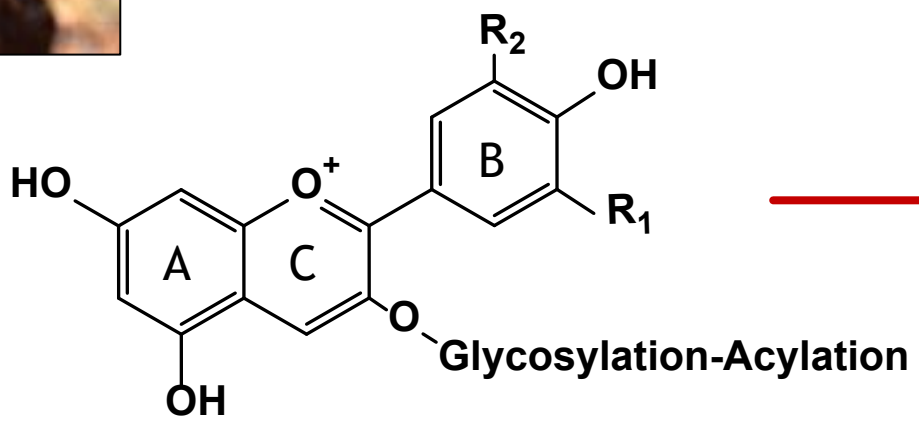
Will this stain ever fade?

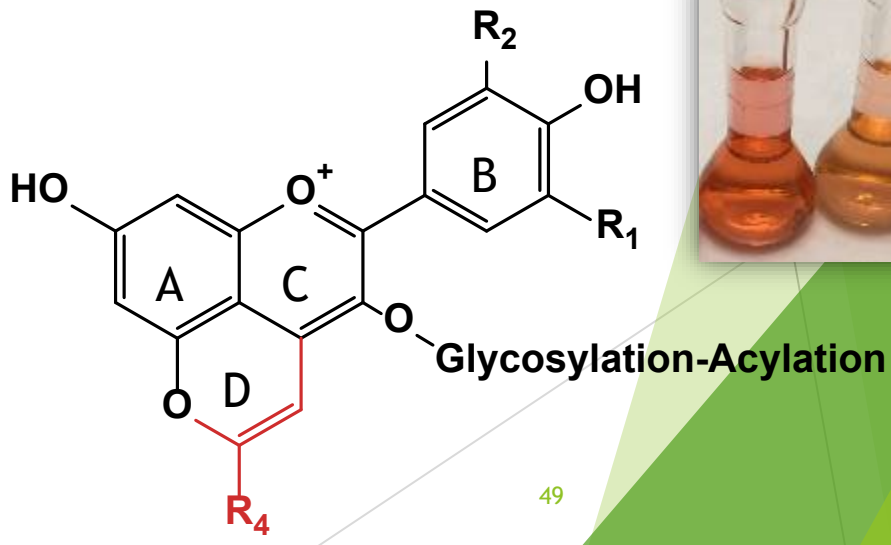


Pigments in Wine

Young wine:
Anthocyanins[1] from grapes

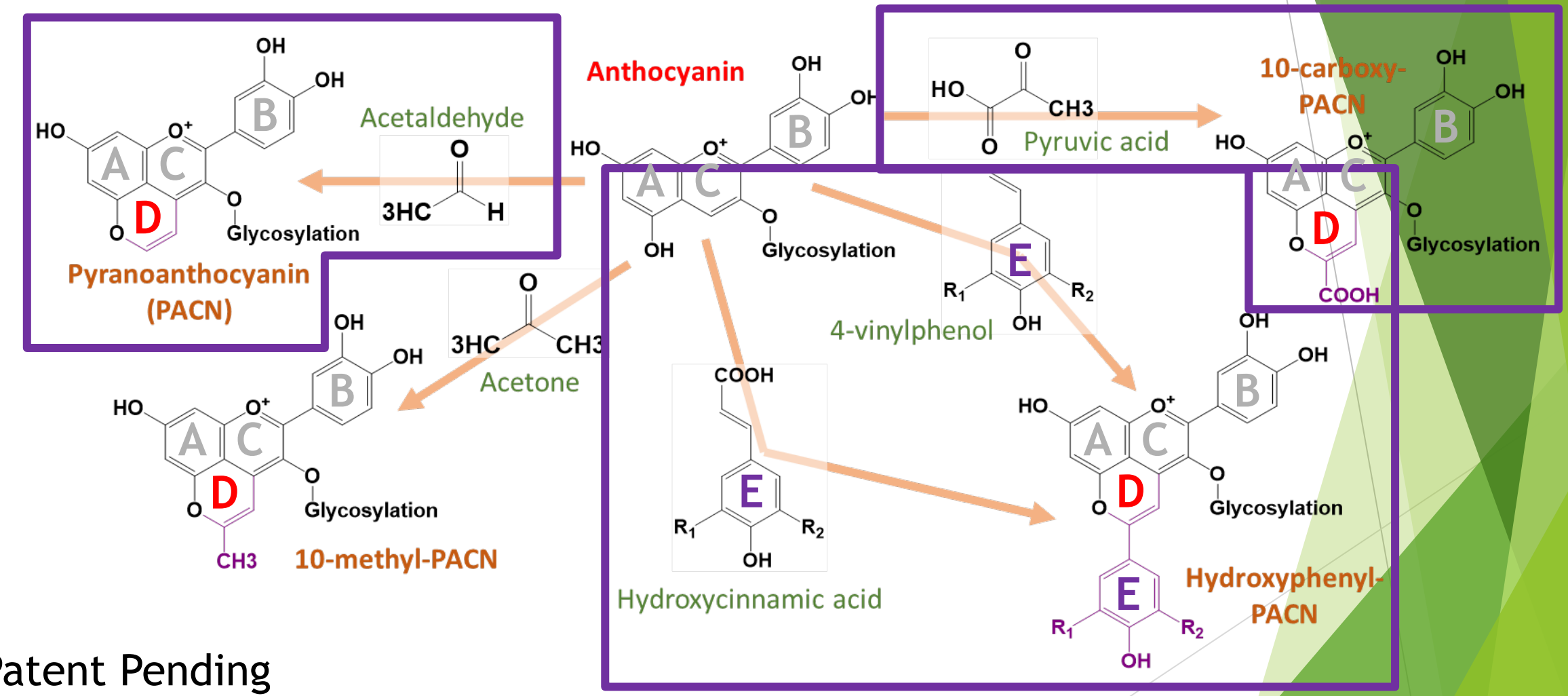
Aged wine:
Pyranoanthocyanin
- Formed during wine fermentation[1]
- Higher stability[2]





1. Sigurdson, G., Tang, P., Giusti, MM. (2017). *Annu. Rev. Food Sci. Technol.* 8:261-80
2. Sun, J., Li, X., Luo, H. *et al.* (2020). *J. Agric. Food Chem.* 68: 2783-94

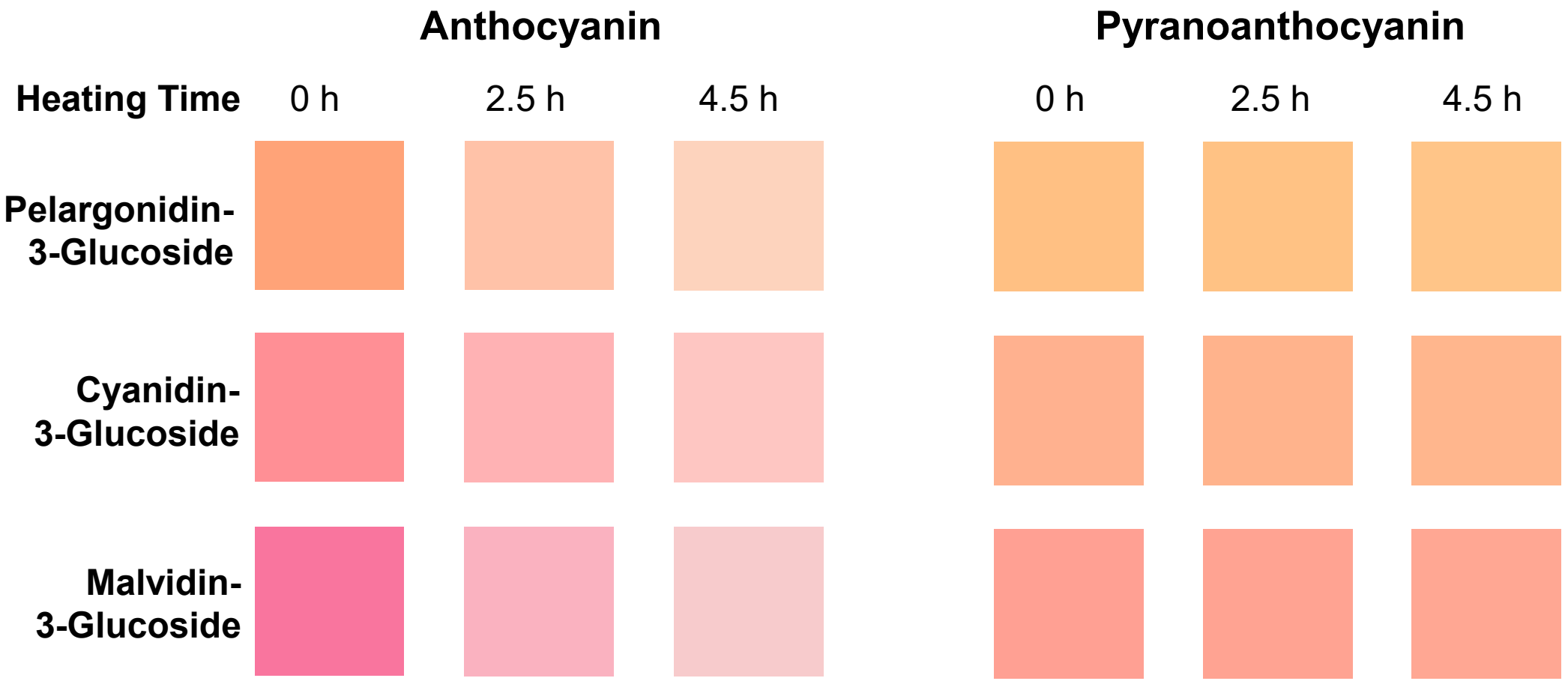
Types of Pyranoanthocyanins (PACN)



Patent Pending

Zhu, X, **Giusti, MM**. 2021. PACN formation by Cyanidin and pyruvic or caffeic acids. Food Chem. 345:128776. Miyagusuku-Cruzado, G; Voss, DM; Giusti, MM. 2021. Anthocyanin & Cofactor Structure on PACN formation Efficiency. Int. J. Mol. Sci. 2021, 22, 6708.

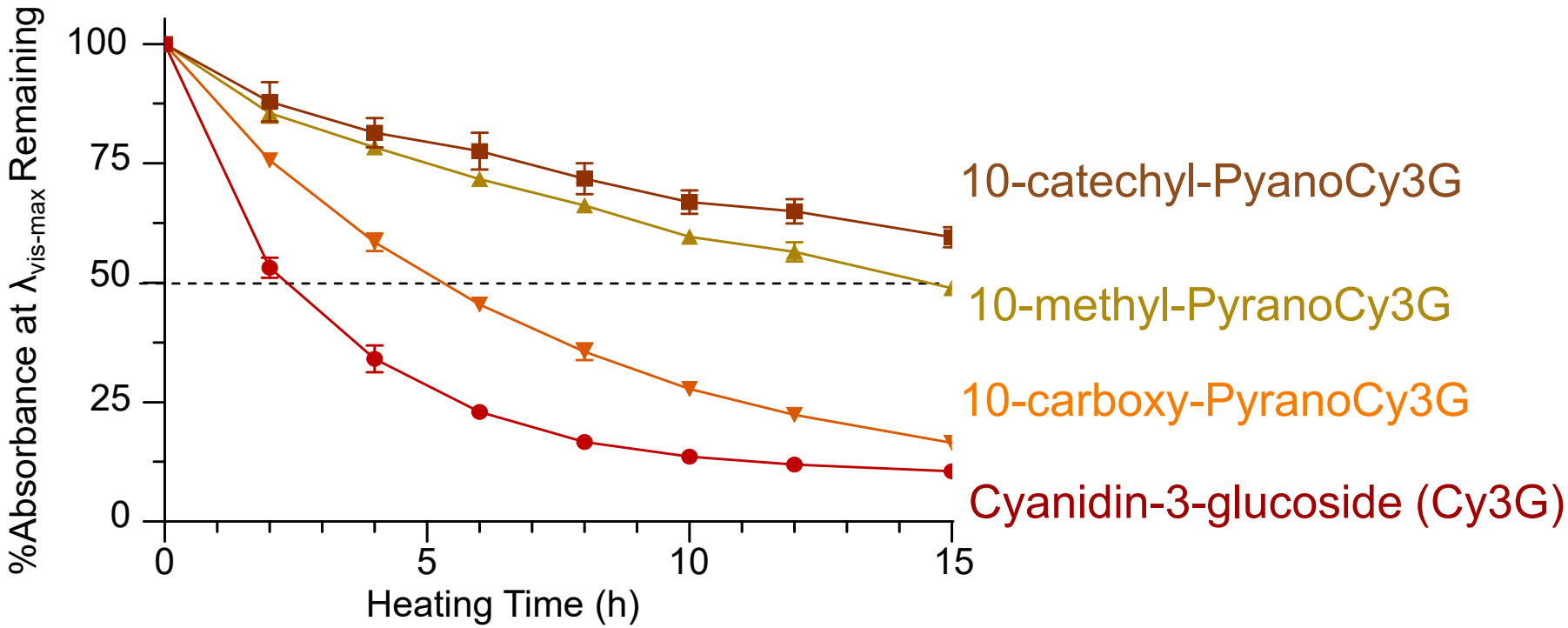
Pyranoanthocyanins: Higher Heat Stability



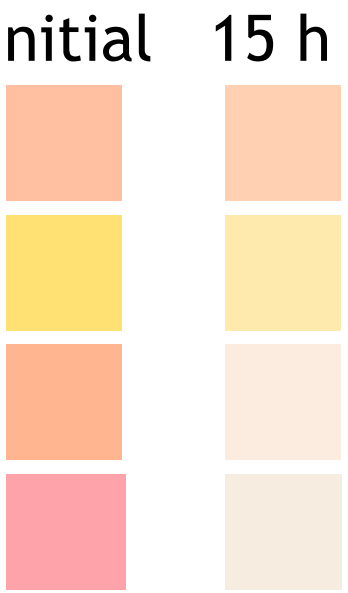
Color swatches represent CIE L*a*b* values of heated pigmented solutions (pH 3.0) based on visible light absorbance spectra.

Farr, JE; **Giusti, MM** (2018). Investigating the interaction of ascorbic acid with anthocyanins and pyranoanthocyanins. *Molecules* 23 (4), 744-744.

Heat Stability of Color



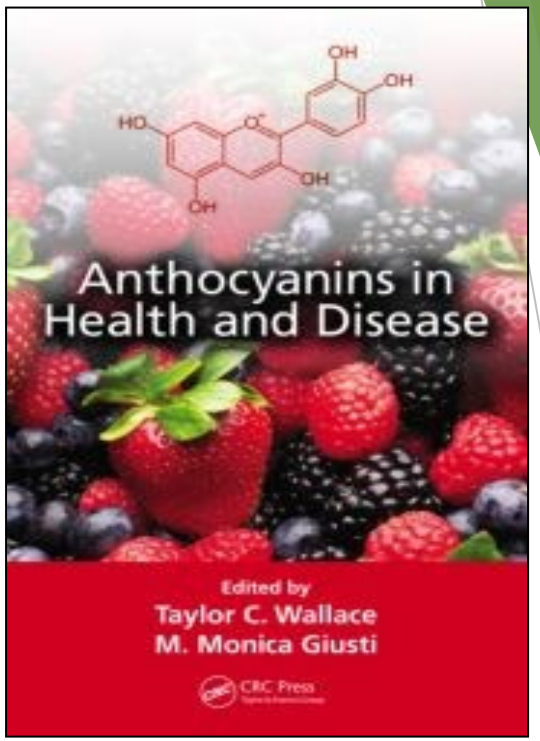
Color changes with heat



- Pyranoanthocyanins were 2–9X more stable than anthocyanins with 90°C heat
- 10-Catechyl-PyranoCyanidin-3-Glucoside had the most stable color

Voss, DM; Miyagusuku-Cruzado, G; Giusti, MM (2022). Thermal stability of 10-carboxy-, 10-methyl-, and 10-cathechyl-PACN. npj Science of Food, 6:

Anthocyanin Bioavailability and Bioactivity



- Anthocyanin stability in the GIT
  - Starting from the oral cavity
- Chemoprotective effects of anthocyanins
- Anthocyanin penetration in the skin - cosmetics

He, J; Giusti, MM. Health promoting Anthocyanins Ann Rev. Food Sci Technol. 1 (1): 163-187. Kamonpatana, K; **Giusti, MM**; (…) Failla, ML. 2012. Anthocyanins in human saliva. *Food Chem.* 135: 738-747. Singletary, KW; Jung, KJ; Giusti, MM. Anthocyanin extract blocks DNA damage. *J. Med. Foods*.10 (2) 244-251. Westfall, A, (…), Giusti, MM. 2020. Penetration of topically applied anthocyanins. Antioxidants 9 (6) 486, 1-14.

Some Final Considerations

- Universal color solutions do not exist
- Creating new products will be easier than color matching old formulations
  - Some changes in the process may be needed

Transitioning from synthetics can be challenging

Artificial vs Natural Colors

…Some Final Considerations

- Work with suppliers you trust
  - Colorant companies will work with you!
  - Solutions will be based on application and needs
- Costs may increase, but customers may be willing to pay more
- Colors from nature may provide more than color
  - Health benefits?
- There is plenty to learn in this fascinating field

Thank you!

https://u.osu.edu/giustilab/

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