Lesson Plan: Investigating the pH Sensitivity of Natural Food Colors

Grade Level: High School Chemistry

Lesson Duration: 90 minutes

NGSS Standards:

- **HS-PS1-2**: Construct and revise an explanation for the outcome of a simple chemical reaction based on the outermost electron states of atoms, trends in the periodic table, and knowledge of the patterns of chemical properties.
- **HS-PS1-5**: Apply scientific principles and evidence to explain how the rate of chemical reactions is influenced by changes in conditions.
- **HS-ETS1-3**: Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics.

Lesson Objectives

By the end of the lesson, students will be able to:

1. Explain why natural food colors like beetroot and cranberry extracts change color in different pH environments.
2. Identify the chemical properties of betalains (the pigments in beets) and anthocyanins (the pigments in cranberries) and their role as pH indicators.
3. Conduct an experiment to observe color changes of natural food colors with various acids and bases.
4. Relate their findings to real-world applications of natural pH indicators.

Background and Introduction

It is said that we eat with our eyes, meaning that we rely on visual cues to identify food safety, freshness, and palatability. Food dyes are often used to enhance food appearance and desirability. These dyes can come from both natural and synthetic sources. Red dye #3 is a synthetic dye that was banned as a food additive in the US in 2025 (effective 2027), but fifty-three years earlier red dye #2 was banned. At that time, there were not a lot of natural color options available for food manufacturers to enhance the appearance of their foods. Two researchers at the University of Wisconsin-Madison set about solving that problem, and they recovered value from wasted food materials at the same time.

Wisconsin and New York regularly trade places for the nation's top producer of table beets. These root vegetables are notorious for their deep red color. While they can be consumed fresh, most harvested beets are canned. The canning process necessarily involves submerging beets in water, at which time they leach significant amounts of their red pigments. A UW-Madison horticulture professor, Warren “Buck” Gabelman, noticed the waste of all this pigment while he was working on breeding better beets and touring canning facilities across the state in the 1970s. He collaborated with a UW-Madison food science professor, Joe von Elbe, who developed an evaporative method to remove water and concentrate this previously wasted pigment down to 66% solids. This concentrated pigment was then able to dye foods a range of red-pink colors. Further enhancing the work, Buck bred beets to increase their pigment concentration, allowing for more color to be collected from each harvest.

Beetroots owe their deep red color to a group of pigments known as betalains, including both red betacyanins and yellow/orange betaxanthins (**Fig. 1**). These pigments are water-soluble and can degrade with heat, light, and pH fluctuations. Research from Buck, Joe, and like-minded researchers helped uncover the molecular structure and stability of betalains, contributing to a better understanding of how these pigment compounds behave in different environments. For instance, betalain pigments experience minor hue shifts at acidic to neutral pH (3-8, **Fig. 2A**). Thus, betalains provide more stability than similar-hued natural colorants, anthocyanins, which experience significant color changes under the same conditions (**Fig. 2B**). Anthocyanins are found in a wide range of red- to purple-hued foods including cranberries, red cabbage, and grapes. (Fun fact: cranberries are the Wisconsin state fruit, and we produce about 60% of all U.S. cranberries!) Knowledge of natural color stability is important for selection of appropriate colorants in food manufacturing, where the pH range 3-8 encompasses a large majority of foods.

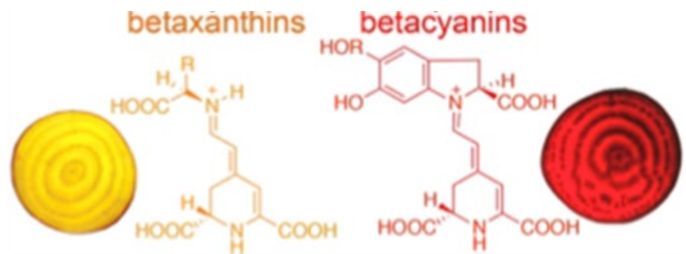

Figure 1. Structure of two betalain compounds.

Like litmus used in traditional litmus paper pH indicators, betalains and anthocyanins shift colors as the concentration of hydrogen ions (H^+) changes (**Fig. 2**). Chromophores are parts of pigment compounds that absorb particular light wavelengths and reflect others, thus we observe the reflected wavelengths as color. The pigment compounds have various functional groups that can be altered by pH shifts, that is by the concentration of hydrogen ions (H^+) in the environment. If these functional groups are present on chromophores, then the pH shift may alter the color observed largely based on the functional group’s pKa. As an example, carboxylic acid groups have a pKa of 4. So, below pH 4, carboxylic acid would be protonated, but above pH 4 carboxylic would be deprotonated (**Fig. 3**). This change would alter the color of the pigment if the carboxylic acid was present on the chromophore of a pigment compound. Multiple chromophores and multiple functional groups lead to pigment compound producing a rainbow of colors across the pH spectrum. At low pH (acidic conditions), beet pigments appear reddish-pink, while at high pH (basic conditions), they transition to purple or yellowish-brown. This pH-dependent color change is due to structural modifications of the chromophores in the betalain molecules, affecting how they absorb and reflect light (**Fig. 4A**). Anthocyanins similarly change color with pH shifts due to structural changes (**Fig. 4B**).

Thanks to the work Buck and Joe started in the 1970's, vibrant red beet pigments are currently available to replace synthetic colors like the recently banned red dye #3. In fact, you can find products colored by beets at your local supermarket today (check the ingredient statements for "beet juice concentrate")! Researchers at UW-Madison are still advancing beets – for example, Irwin Goldman is developing beet varieties with less "earthiness" flavor to enhance their palatability.

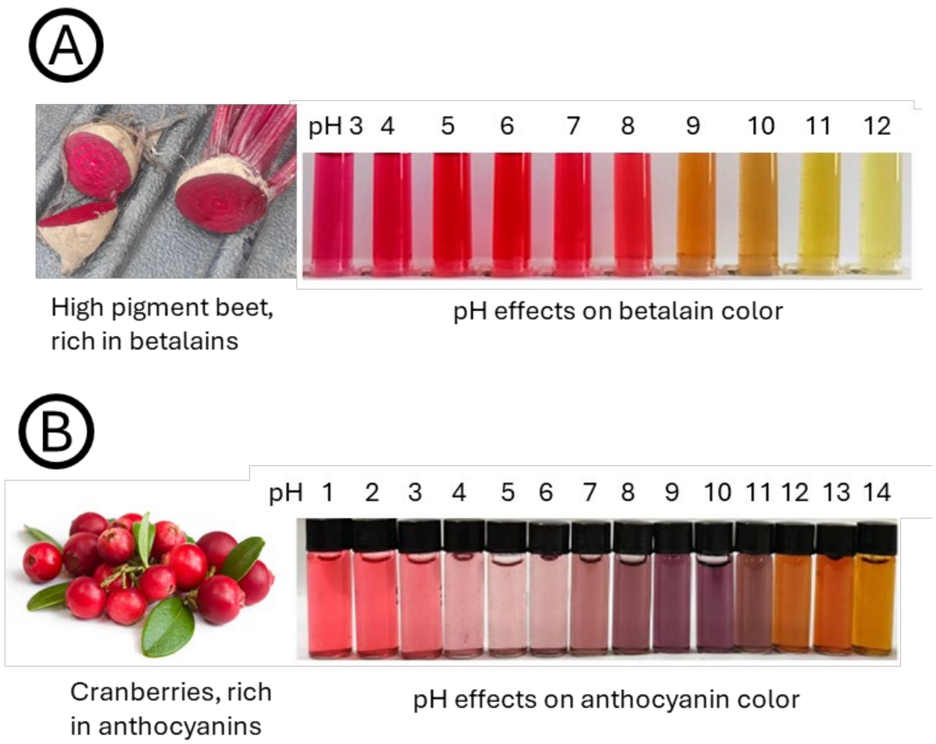

Figure 2. Examples of pH effects on natural pigments. A: Betalains, found in foods like beets. B: Anthocyanins, found in foods like cranberries.

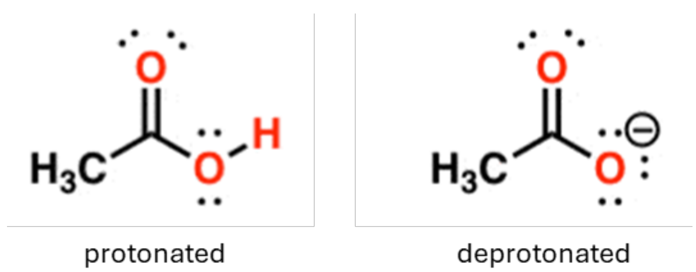

Figure 3. Protonated versus deprotonated carboxylic acid.

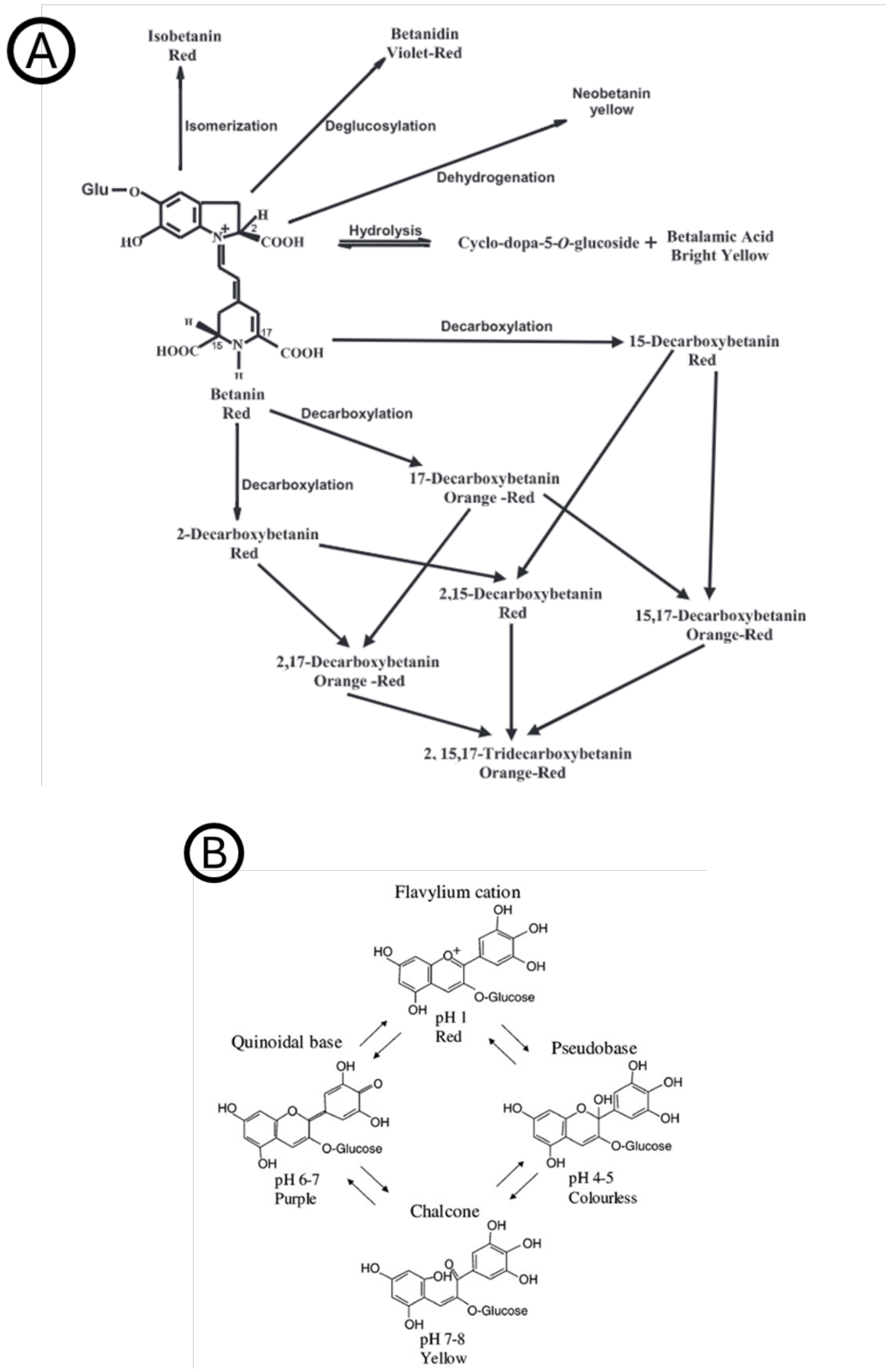

Figure 4. Schematics of chemical alterations to (A) betacyanin and (B) anthocyanin structures.

Lesson Structure

1. Engagement (10 minutes) – Hook & Introduction

- **Demonstration:**
  - Show students two beakers: one with acidic lemon juice and one with a basic solution (e.g., ammonia or other solution with pH > 10).
  - Add beetroot extract to both solutions and observe the immediate color changes.
- **Discussion Questions:**
  - Why do you think the color is changing?
  - What does this tell us about the chemical properties of beetroot pigment?
  - Where else might pH indicators be useful?

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2. Exploration (25 minutes) – Hands-On Experiment

Materials Needed:

- Beetroot extract
  - Prepare by microwaving fresh beets to soften, blending with water (1:1 weight with beets), then straining through cheese cloth. *NOTE: The method below was developed by diluting a very concentrated beetroot solution. You may have to adjust dilution of your beetroot extract.*
  - *Special note:* If using beetroot extract sample acquired at WSST 2025 – These samples are labeled "B" and should be diluted 1:49 in water.
- Cranberry extract
  - Prepare by microwaving fresh cranberries to soften, blending with water (1:1 weight with beets), then straining through cheese cloth.
  - *Special note:* If using cranberry extract sample acquired at WSST 2025 – These samples are labeled "C" and should be diluted 1:9 in water.
- pH buffer solutions (pH 2, 4, 6, 8, 10, 12). Alternatively, you could use household acids/bases (vinegar, lemon juice, baking soda, ammonia, etc.) to produce a range of pH.
- Test tubes
- pH test strips (for validation)
- Dropper pipettes
- Lab goggles and gloves

Procedure:

1. Pour 10mL of each different buffer solution or household acid/base into different test tubes for a total of six test tubes.
2. Using a transfer pipette, add 5 drops of beetroot extract into each tube.
3. Observe and record color changes.
4. Use pH test strips to verify the pH of each solution.
5. Repeat steps 1-4, but substitute cranberry extract for beetroot in step 2.
6. Compare results and document findings.

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3. Explanation (20 minutes) – Conceptual Understanding

- **Discussion of Results:**
  - Students compare their color observations to a pH scale and identify trends.
  - Explain that betalains (pigments in beetroot) and anthocyanins (the pigments in cranberries) change structure depending on the hydrogen ion concentration, leading to color variation.
  - Relate findings to commercial pH indicators like litmus paper.
  - Discuss how research at UW-Madison on beet pigment concentration and characterization helped industry develop natural dyes. Ideate ways for scientists to further develop more stable natural dyes and food colorants from beets, cranberries, or other sources.

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4. Elaboration (25 minutes) – Real-World Applications & Deeper Thinking

- **Research & Discussion:**
  - Identify the pH of lemonade (2.5) and of milk (6.9) either by testing in the lab or through students guessing. Have students predict the colors you would observe if beetroot or cranberry extract were used to color these beverages.
  - Discuss how pH indicators are used in food safety, medicine, and environmental monitoring.
    - E.g., pH indicators can be incorporated into food packaging to indicate food freshness related to pH. See Table 4 (below) from Roy & Rhim, 2021, Critical Reviews in Food Science & Nutrition, https://doi.org/10.1080/10408398.2020.1776211

Table 4. Anthocyanin incorporated other polymers/biopolymers-based smart packaging films for food packaging applications.

Source of anthocyanin	Source of polymer	Application	Reference
Red cabbage (*Brassica oleraceae*) extract	Chitosan, gelatin, PVA	Smart packaging of ricotta cheese spoilage	Bento et al. (2015)
Mulberry anthocyanin	Ethylene-vinyl alcohol copolymer	Shrimp freshness monitoring	Kang et al. (2018)
Purple cabbage extract	Dimethyl acrylamide/Gelatin	Smart food packaging	Alpaslan, Dudu, and Aktaş (2018)
Red cabbage	Fish gelatin	Intelligent food packaging films	Uranga et al. (2018)
Mulberry extract	PVA	Monitoring the spoilage of fish	Ma et al. (2018)
Red cabbage (*Brassica oleraceae*) extract	Bacterial cellulose nanofibers	Smart food packaging films	Pourjavaher et al. (2017)
Red cabbage extracts	Gelatin	Smart food packaging films	Musso, Salgado, and Mauri (2019)
Mulberry anthocyanin extracts	Gelatin/PVA	Monitoring raw mud carp (*Cirrhina molitorella*) freshness	Zeng et al. (2019)
Rosemary extract	Furcellaran/gelatin hydrolysate	Fish spoilage test	Jancikova et al. (2019)
Rosemary extract	Ethylene-vinyl acetate	Smart food packaging films	Eskandarabadi et al. (2019)
Prunus maackii extract	κ-carrageenan/hydroxypropyl methylcellulose	To monitor pork spoilage	Sun, Chi, et al. (2020)
Purple sweet potato	Cellulose nanofiber	Intelligent packaging	Chen, Zhang, et al. (2020)
Red radish	Gelatin/cornstarch	Monitoring meat spoilage, whole white prawns (*Fenneropenaeus merguiensis*) and chicken meat (*Gallus gallus domesticus*)	Chayavanich, Thiraphibundet, and Imyim (2020)
Rose anthocyanins	PVA/okra mucilage polysaccharide	Monitoring freshness of Shrimp	Kang et al. (2020)
Rosemary	PVA/starch	Monitoring milk spoilage	Mustafa et al. (2020)
Red cabbage, sweet potatoes, roselle, butterfly pea, mangosteen, and red dragon fruit	bovine gelatin	Smart food packaging films	Rawdkuen et al. (2020)
Purple sweet potato	PVA/starch	Monitoring bighead carp (*Hypophthalmichthys nobilis*) freshness	Chen, Zhang, et al. (2020)

- Explore how researchers are studying ways to stabilize beet pigments for commercial applications.
  - E.g., encapsulation to shield from pH changes, light, heat. See more in Rodriguez-Amaya, 2019, *Food Research International,* https://doi.org/10.1016/j.foodres.2018.05.028
- Challenge students to think about other natural colors and pH indicators in food (e.g., red cabbage, turmeric, blueberries) and enumerate various ways they could be applied. (Option: have lab groups pair up, work through this, then share findings with the class.)

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5. Evaluation (10 minutes) – Assessment & Reflection

- **Exit Ticket Questions:**
  1. Explain why beetroot and cranberry extracts change color when exposed to acids and bases.
  2. How does this experiment demonstrate the effect of pH on chemical compounds?
  3. What did researchers at UW-Madison discover about the stability of beet pigments?
  4. Give one real-world example where a natural pH indicator could be useful.
- **Optional:** Lab report summarizing their experiment and findings.

Differentiation & Extensions

- **For Advanced Students:** Research the molecular structure of betalains or anthocyanins and write a short report on how their chromophores interact with hydrogen ions.
- **For Struggling Students:** Provide a color-coded pH chart for reference and allow group discussions for better understanding.
- **Extension Activity:** Have students test the pH of different foods and drinks at home using beetroot or cranberry extract, or other natural pH indicator.

Conclusion

This lesson provides an engaging, hands-on approach to understanding pH indicators using natural substances, reinforcing chemistry concepts while connecting to real-world applications. It also introduces students to scientific research conducted at UW-Madison, showing how chemistry is used to solve real-world problems in food science, sustainability, and health.

Student Lab Worksheet: Investigating the pH Sensitivity of Natural Food Colors

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Lesson Objectives

By the end of the lesson, students will be able to:

1. Explain why natural food colors change color in different pH environments.
2. Identify the chemical properties of natural colors and their role as pH indicators.
3. Conduct an experiment to observe color changes of natural food colors with various acids and bases.
4. Relate their findings to real-world applications of natural pH indicators.

Background Reading

Foods like fruits, vegetables, and beans present a rainbow of colors. These vibrant-colored foods can stain your hands and teeth as you eat them (think of raspberries, beets, and spinach). The pigment compounds that color these foods and stain you can be extracted and used as food dyes. However, one of the challenges of using natural colorants is their stability during food processing and storage. Most natural colorants degrade with heat, light, and pH fluctuations. Imagine baking a cake that is dyed red with cranberry juice – the pH of the batter and the heat of the oven during baking are likely to alter the color. In this lab, we will explore the stability of two natural colorants to pH changes.

Chromophores are parts of pigment compounds that absorb particular light wavelengths and reflect others, thus we observe the reflected wavelengths as color. The pigment compounds have various functional groups that can be altered by pH shifts, that is by the concentration of hydrogen ions (H^+) in the environment. If these functional groups are present on chromophores, then the pH shift may alter the color observed based on the functional group's pKa. As an example, carboxylic acid groups have a pKa of 4. So, below pH 4, carboxylic acid would be protonated, but above pH 4 carboxylic would be deprotonated (**Fig. 1**). This change would alter the color of the pigment if the carboxylic acid was present on the chromophore of a pigment compound. Multiple chromophores and multiple functional groups lead to pigment compound producing a rainbow of colors across the pH spectrum.

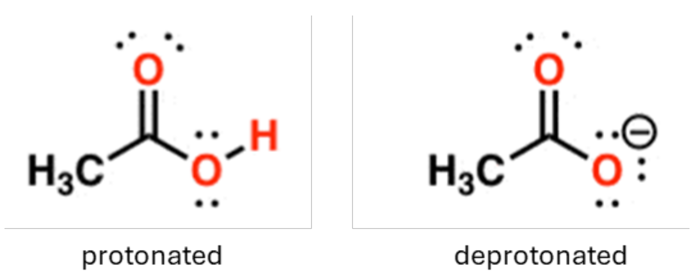

Figure 1. Protonated versus deprotonated carboxylic acid.

Pre-Lab Questions

1. A given chemical structure extracted from a food provides a bright red color at pH 3 but a bright blue color at pH 7. Based on the background reading, what is happening to produce this color change?
2. Have you ever used one food (like fruits or vegetables) to dye another (like hard-boiled eggs or lemonade)? If so, describe the foods and colors. If not, think of a fruit that you might use to dye lemonade and predict the color.

Materials and Procedure

<u>Materials</u>

- Beetroot extract
- Cranberry extract
- pH solutions (pH 2, 4, 6, 8, 10, 12)
- Test tubes (12)
- pH test strips (for validation)
- Dropper pipettes
- Lab goggles and gloves

<u>Procedure</u>

1. Pour 10mL of each different pH solution into different test tubes for a total of six test tubes.
2. Using a transfer pipette, add 5 drops of beetroot extract into each tube.
3. Observe and record color changes and anything else you notice about the samples.
4. Use pH test strips to verify the pH of each solution.
5. Repeat steps 1-4, but substitute cranberry extract for beetroot in step 2.
6. Compare results and document findings.

Results

Natural Food Color:

pH	Color Observed	Notes

Natural Food Color:

pH	Color Observed	Notes

Other observations:

Discussion Questions

1. Explain why beetroot and cranberry extracts change color when exposed to acids and bases.
2. How does this experiment demonstrate the effect of pH on chemical compounds?
3. If you were using a cake frosting dyed with cranberry extract, how could you produce a range of colors without changing food dyes?

Post-Lab Reading & Reflection

It is said that we eat with our eyes, meaning that we rely on visual cues to identify food safety, freshness, and palatability. Food dyes are often used to enhance food appearance and desirability. These dyes can come from both natural and synthetic sources. Red dye #3 is a synthetic dye that was banned as a food additive in the US in 2025 (effective 2027), but fifty-three years earlier red dye #2 was banned. At that time, there were not a lot of natural color options available for food manufacturers to enhance the appearance of their foods. Two researchers at the University of Wisconsin-Madison set about solving that problem, and they recovered value from wasted food materials at the same time.

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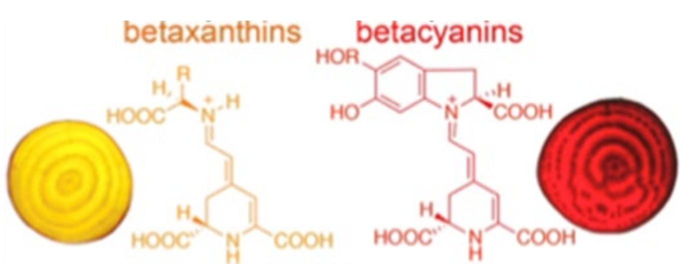

Figure 1. Structure of two betalain compounds.

Like litmus used in traditional litmus paper pH indicators, betalains and anthocyanins shift colors as the concentration of hydrogen ions (H^+) changes (**Fig. 2**). At low pH (acidic conditions), beet pigments appear reddish-pink, while at high pH (basic conditions), they transition to purple or yellowish-brown. This pH-dependent color change is due to structural modifications of the chromophores in the betalain molecules, affecting how they absorb and reflect light (**Fig. 3A**). Anthocyanins similarly change color with pH shifts due to structural changes (**Fig. 3B**).

Thanks to the work Buck and Joe started in the 1970's, vibrant red beet pigments are currently available to replace synthetic colors like the recently banned red dye #3. In fact, you can find products colored by beets at your local supermarket today (check the ingredient statements for "beet juice concentrate")! Researchers at UW-Madison are still advancing beets – for example, Irwin Goldman is developing beet varieties with less "earthiness" flavor to enhance their palatability.

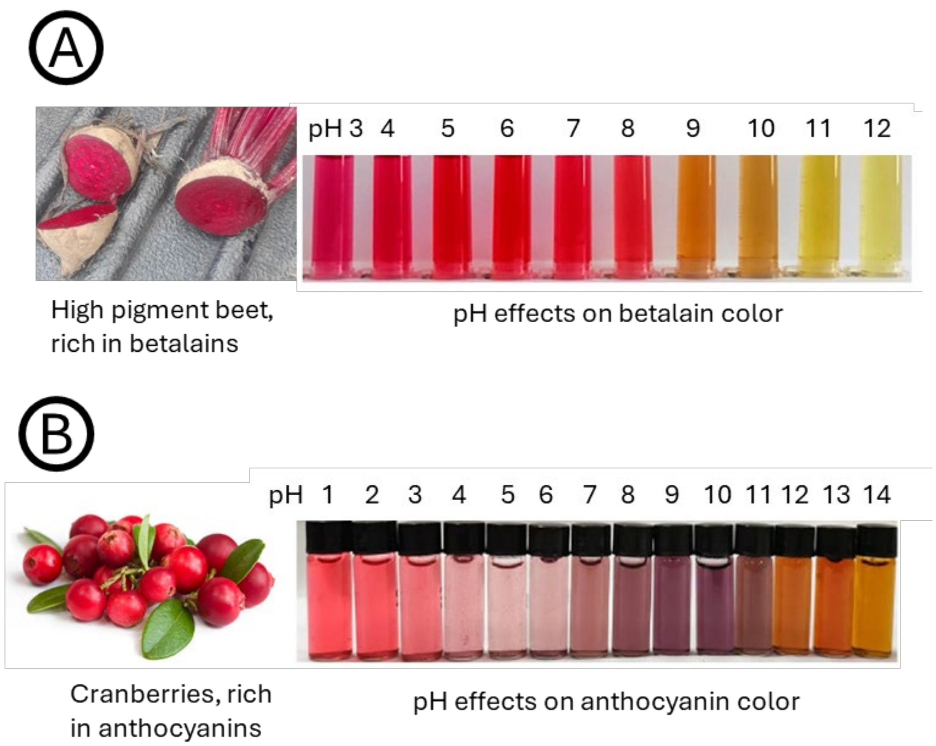

Figure 2. Examples of pH effects on natural pigments. A: Betalains, found in foods like beets. B: Anthocyanins, found in foods like cranberries.

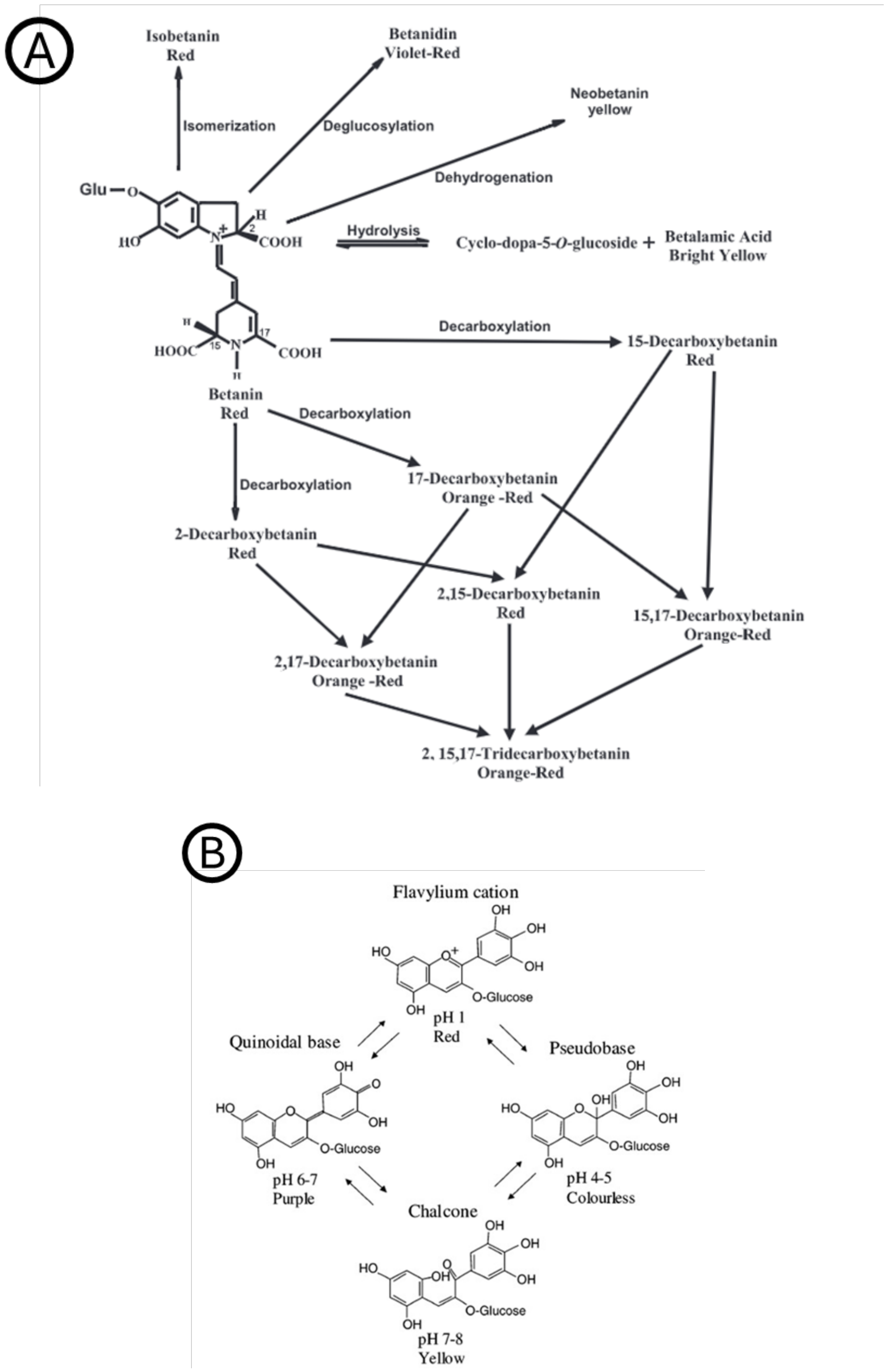

Figure 3. Schematics of chemical alterations to (A) betacyanin and (B) anthocyanin structures.

Reflection Questions

1. Select another highly pigmented fruit or vegetable. Consider where during food processing it might produce wasted material (e.g., beet peels, canned beet juice, beet tops, etc.). Can you think of a way to recover natural colorants from this material?
2. Choose one of the chemical reactions listed in Fig. 3A that lead to a betacyanin color change. Explain how pH alterations are important in this chemical reaction.
3. Describe one application of natural food colorants as pH indicators.