May 10, 2024

Food grade colorimetry of anthocyanins (A Youth Summer Camp Activity)

DOI
dx.doi.org/10.17504/protocols.io.yxmvmenqng3p/v1

Eric S McLamore[1,2], Geisianny AM Moreira[1,3], Diana Vanegas[3], dbahamo[3], Lisseth Casso-Hartmann[3], Lidadi Agbomi[1,2], Maria J Torres[4]

[1]1Department of Agricultural Sciences, Clemson University;
[2]2Department of Earth and Environmental Sciences, Clemson University; [3]3Biosystems Engineering, Clemson University;
[4]5Department of Plant and Environmental Sciences, Clemson University

Eric S McLamore: corresponding author;

Eric S McLamore
Clemson University, North Carolina State University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.yxmvmenqng3p/v1

Protocol Citation: Eric S McLamore, Geisianny AM Moreira, Diana Vanegas, dbahamo, Lisseth Casso-Hartmann, Lidadi Agbomi, Maria J Torres 2024. Food grade colorimetry of anthocyanins (A Youth Summer Camp Activity). **protocols.io**
https://dx.doi.org/10.17504/protocols.io.yxmvmenqng3p/v1

Protocol status: Working
We use this protocol and it's working

Created: May 08, 2024

Last Modified: May 10, 2024

Protocol Integer ID: 99412

Funders Acknowledgement:
NIFA Agriculture and Food Research Initiative
Grant ID: 2018-67016-27578
National Science Foundation
Grant ID: CBET: 2019435

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Food and Agriculture nor the National Science Foundation.

Abstract

This protocol describes activities used in summer education camps for youth (14 years and older). The activities involve extraction and cryo-concentration of plant pigment (anthocyanin) from red cabbage. This protocol describes summer camp activities for encapsulation/spherification in alginate beads and other alternative methods for teaching basic principles related to cross linking and colorimetry.

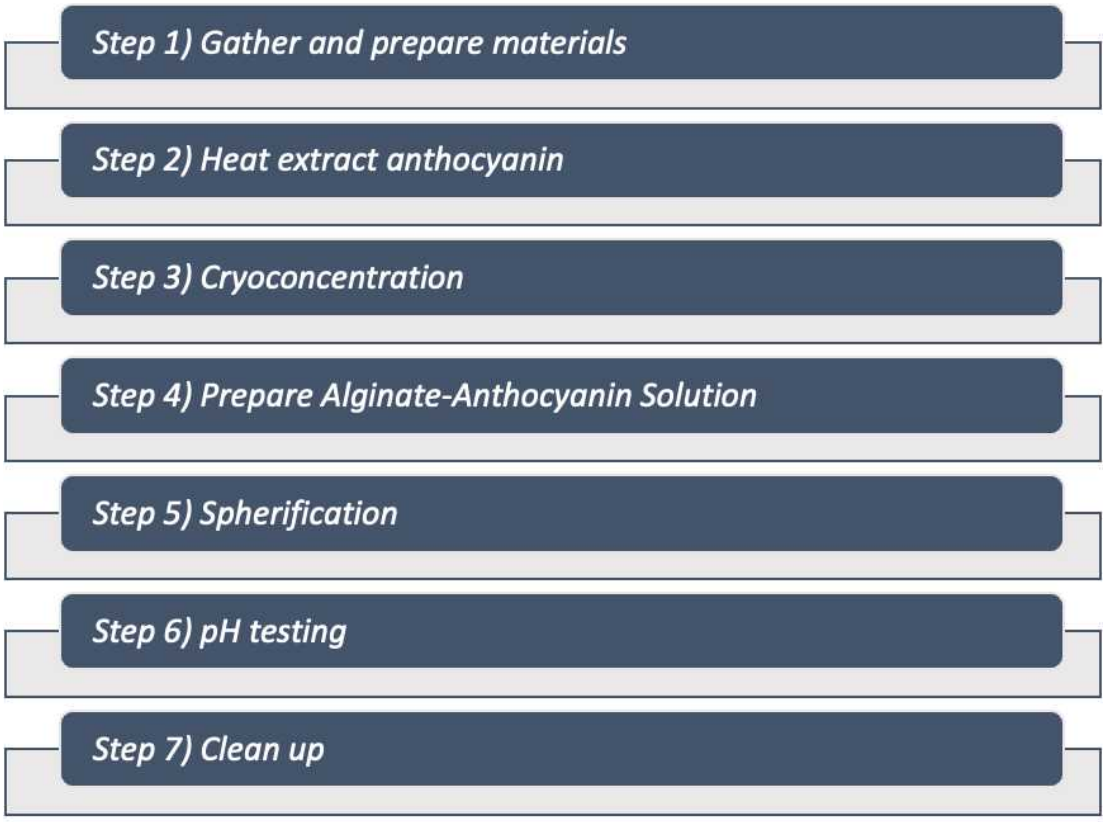

Figure 1. Process flow for cabbage bio-bead exercise. Steps 1-4 may be completed prior to camp activities to maximize
efficiency.

Materials

Hardware

- 66 Quart Stainless Steel pot (**link**)
- Vegetable cleaver (**link**)
- Large strainer or colander (**link**)
- Freezer (0° F)
- ***Optional***: 500W Stainless Steel Immersion Blender (**link**)
- ***Optional***: Colorimeter (**link here**)

Software

- ***Optional***: Color Name AR app (**link here**)

Chemicals, Reagents and Other Materials

- Food grade calcium chloride (or food grade) (**link to MSDS**)
- Food grade calcium lactose (**link to MSDS**)
- Food grade sodium alginate (**link to MSDS**)
- Sodium bicarbonate (baking soda) from a local grocer (**link**)
- 50mL Falcon tubes for freezing extract (**link**)
- Red cabbage (*Brassica oleracea var. capitata, F. rubra*) purchased from a local grocer. One medium sized head of cabbage is approximately 2 lbs (900 g)
- White distilled vinegar from a local grocer
- Small plastic tubs/dishes with lids (4 tubs per team) from a local grocer
- Plastic spoons from a local grocer (at least 4 per team)
- Plastic weigh boats (at least 4 per team)
- Transfer pipettes (at least 4 per team)

Safety warnings

Eye protection

- Laboratory eye protection or equivalent personal protective equipment (PPE) is required for all activities.
- ***Calcium chloride*** can cause serious eye irritation. If exposure occurs, rinse cautiously with water for several minutes. Remove contact lenses. If eye irritation persists for more than 24 hours, seek medical advice/attention from an ophthalmologist.
- If any ***powders or white vinegar*** contact eye, remove contact lenses and flush eyes with bottled water (or other sterilized water) for at least 15 minutes, occasionally lifting the upper and lower eyelids. If eye irritation persists for more than 24 hours, seek medical advice/attention from an ophthalmologist.

Skin

- If ***calcium lactate*** contacts bare skin, immediately remove nearby contaminated clothing or footwear. Wash skin with plenty of water.
- If any other ***powders or white vinegar*** contact skin, immediately flush skin with soap and water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing with laundry detergent before reuse.
- Caution should be used when cutting/chopping cabbage with a knife

Fumes/aerosols/inhalation

- If any ***powders*** are inhaled, remove person to fresh air and keep comfortable for breathing.

Ingestion

- If any powders are ingested, DO NOT induce vomiting. Drink plenty of water. If any symptoms are noted, including upset stomach, call a POISON CONTROL CENTER or doctor/physician.
- Once food grade powders are solvated with water and prepared according to manufacturers instructions, materials are safe for ingestion.

- *Heat and Flammable Materials*
  Care must be taken with hot liquids and cooking equipment surfaces if extracting the pigment from plant leaves. To avoid potential injury, it is recommended to complete this task at least two days prior to planned event.
- Sodium bicarbonate powder should never be heated in absence of water. When heated to decomposition, this emits acrid smoke, fumes, carbon dioxide and sodium oxides which are all potentially hazardous.

Disposal

- All powders, gels and liquid materials are food grade and may be either composted or disposed in the standard trash receptacle.
- Used 50mL Falcon tubes and transfer pipettes may be reused after rinsing in warm water with soap.

Ethics statement

N/A

Before start

Before starting

- For most applications, it is recommended to heat extract the anthocyanin at least two days prior to events, allowing at least one day to freeze the extract.
- If the anthocyanin is heat extracted during activities, ensure there is ample space between camp participants and cooking equipment. Induction cookware is advised to reduce footprint and limit heat exposure. It is also recommended to perform the heat extraction in a well-ventilated area as not all people enjoy the smell of cooked cabbage!

Note

- Wear eyewear protection at all times.
- Wear gloves and lab coat at all times.

Anthocyanin activities for camp

10h 30m

1 *Step 1) Gather and prepare materials*

30m

- Group participants into teams of 2-4 people (**Fig 1**).

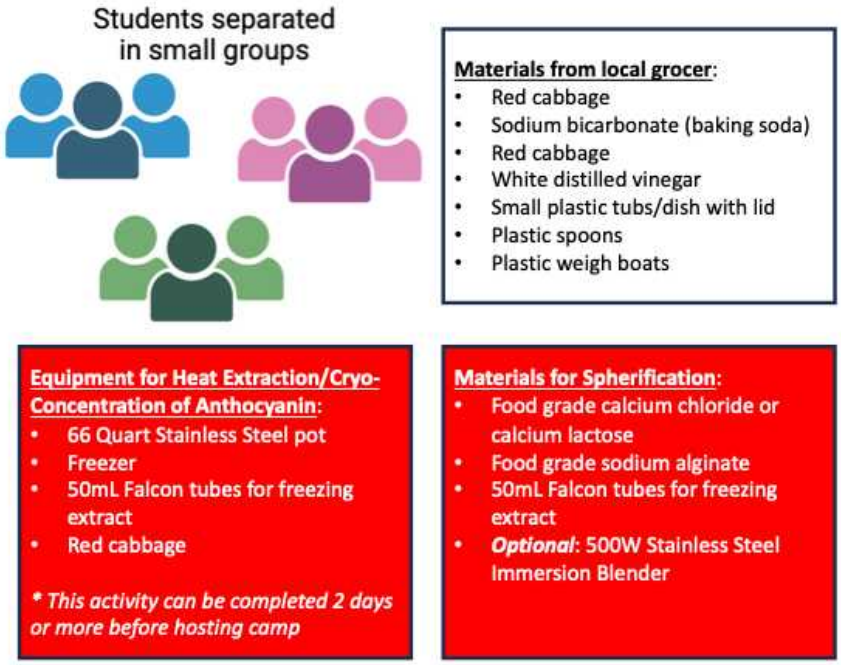

Figure 1. Preparation of materials. The white box shows items which may be obtained at a local grocer. Material/Equipment in the red boxes should be acquired well in advance of camp activities. Equipment for heat extraction/cryo-concentration should be organized at least two days prior to
camp activities.

- **Table 1** presents a planning table for a typical summer camp supporting 40 youth (ten teams of 4). For a camp of this size, 3-5 leaders are typically engaged to guide youth through activities.

	A	B	C
	Material	Example for 40 participants	Note(s)
	66 Quart Stainless Steel pot	1	capacity for 2-3 heads of cabbage
	Red Cabbage	1 head (medium)	1 head of cabbage (approx. 2 lb)
	Pigment Extract	1L	1L of extract per 2 lb of cabbage
	Cryo-Concentrated Extract	100 mL	approximately 100mL of cryoconcentrate may be obtained

	A	B	C
			from 1L of extract
	1% Sodium Bicarbonate solution (food grade)	4 L	1 lb box can produce up to 45L of 1% solution
	1% Calcium Chloride or Calcium Lactose Solution (food grade)	4 L	1 typical (50g) bag of powder can produce up to 5L of solution
	1% Sodium Alginate (food grade)	4 L	1 typical (50g) bag of powder can produce up to 5L of solution
	White Distilled Vinegar	400 mL	128 fl oz. (1 gal) contains 3,785 mL
	Small Plastic Tubs	40	15-20 oz. capacity
	Plastic Spoons	40	Medium (3-4 oz) or large capacity (10-12 oz)
	Plastic Weight Boats	40	3 mL capacity

Table 1. Estimations of materials per participant are shown. The example in the table is for a camp supporting 40 youth (ten teams of four)

Note

It is recommended to heat extract the anthocyanin at least two days prior to events, allowing at least one day to freeze the extract.

The following items are required for heat extraction and cryo-concentration of anthocyanin:

- 66 Quart Stainless Steel pot for boiling cabbage
- Freezer (0° F)
- 50mL Falcon tubes for freezing extract
- Red cabbage (*Brassica oleracea var. capitata, f, rubra*) purchased from a local grocer
- ***Optional***: 500W Stainless Steel Immersion Blender

Note

If this approach is used, the preparation steps below may be completed on the same day as camp activities.

The following items are required for spherification:

- Food grade calcium chloride OR food grade calcium lactose
- Food grade sodium alginate
- Transfer pipettes

- Small plastic tubs/dishes with lids (2 tubs per team)
- Plastic spoons (2 per team)

The following items are required for pH testing:

- Sodium bicarbonate (baking soda)
- White distilled vinegar
- Small plastic tubs/dishes with lids (2 tubs per team)
- Plastic spoons (2 per team)
- ***Optional***: Color Name AR app
- ***Optional***: Colorimeter

2 *Step 2) Heat Extract Anthocyanin* 2h

- Using the vegetable cleaver, cut the cabbage head into quarters.
- Chop leaves into small 1-2 inch wide strips (approximately 3-4 inches long).
- Place Stainless Steel pot on cooking surface.
- Place cabbage strips into pot.
- Fill the pot with water until the cabbage is immersed (approximately 450 g of cabbage for each Liter of water).
- Next, heat the pot until the water boils (approximately 80°C).
- Reduce heat to 75% of maximum level and cook for 120 minutes.
- Check the pot every 30 minutes and stir to avoid burning material on base of pot.
- Turn off heat (cooking equipment).
- Cool down at room temperature for at least 20 minutes but no longer than 60 minutes
- Carefully remove cabbage leaves from the purple liquid extract.
- If possible, a strainer should be used, and the liquid should be collected

Expected result

The purple liquid contains the anthocyanin extract, as well as sugars and other materials. See Demirbas et al 2018 for details

Critical Step:
DO NOT DISCARD PURPLE LIQUID AFTER COOKING!

3 *Step 3) Cryo-Concentrate Anthocyanin* 8h

- Gather the 50mL Falcon tubes.
- Carefully pour approximately 40mL of anthocyanin extract into each tube and then seal.

Note

Do not fill the bottle completely, leave a gap of approximately 1-2 cm between the top of the liquid and the bottle cap to avoid damage during expansion in the freezer.

- Freeze for at least 6 hours (or until a solid ice block forms in each tube).
- After freezing solid, remove the block of frozen extract.
- Place at room temperature for 10 min.
- Remove the lid of the Falcon tube and carefully invert the tube to collect the dark purple syrup (known as “cryo-concentrate”).
- Pour this dark syrup into a separate container. The cryo-concentrate may be combined together, but avoid adding any excess water.
- Approximately 2-4 mL of cryoconcentrated pigment can be acquired for each 40mL vial.

Note

This dark purple syrup has very high levels of anthocyanin.

This can be stored in the freezer indefinitely or in the refrigerator for approximately one week.

Optional: Cook a yummy and spicy meal!

- *For each head of cabbage, chop one full (large) onions, three bell peppers, one bunch of green onions*
- *Obtain one small bag of carrots (optional to chop)*
- *Heat olive oil in a large skillet over medium-high heat; add chopped onions, bell pepper, and green onions.*
- *Cook and stir vegetables until softened, about 5 minutes.*
- *For spicy: Mix in chopped Scotch bonnet pepper,*
- *Add 2 sprigs fresh thyme, and salt as desired*
- *Continue to cook for an additional minute*
- *Stir boiled cabbage and carrots into mixture and cook for an additional 5 min in olive oil*
- *Cover the skillet (or large wok) and reduce heat to medium-low.*
- *Allow to cook for 10 minutes.*
- *While food is cooking, prepare a sauce with ¼ cup white vinegar and 2 tablespoons white vinegar. Stir well, and then add to skillet (or wok)*
- *Cook, uncovered, stirring occasionally, about 2-3 more minutes.*
- *Discard Scotch bonnet pepper and thyme before serving.*

Optional: Cook a yummy and spicy meal!

4 *Step 4) Prepare Alginate-Anthocyanin Solution* 20m

- A total of five solutions should be prepared for this step (**Fig 2**).

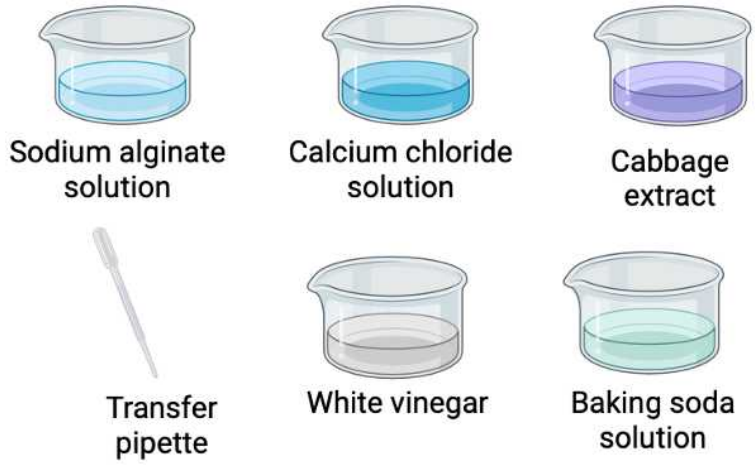

Figure 2. Five solutions should be prepared for this step.

Note

The participants may prepare the solutions if sufficient measurement scales are available during the activity.

Conversely, a larger batch (e.g., 1 to 2 L) of the solution may be prepared in advance.

Solution 1: Prepare a 2% (*w/v*) sodium alginate solution.

- Weigh 2 grams of sodium alginate powder and transfer it to a plastic tub.
- Heat tap water (or bottled water)
- Add 100 mL of hot water to the tub and stir vigorously until no clumps of alginate are present; this process can take up to 10 minutes of stirring.
- ***Optional***: Use an immersion blender to quickly dissolve the alginate in the water.

Solution 2: Prepare 1% (*w/v*) calcium chloride solution. Alternatively, a 1% solution of calcium lactose (*w/v*)
may be used.

- Weigh 1 gram of calcium chloride (or calcium lactose) and transfer it to a plastic tub.
- Add 100 mL of water to the tub and stir until no powder is visible.

Solution 3*:* Prepare a 2% (w/v) solution of sodium bicarbonate.

- Weigh 1 gram of sodium bicarbonate and transfer to a plastic tub.
- Add 100 mL of water to the tub and stir until no powder is visible.

Solution 4: Obtain approximately 4-5 mL of the cryo-concentrate solution from Step 3.

Solution 5*:* Obtain approximately 10mL of white vinegar.

5 *Step 5) Spherification* 15m

- Using a transfer pipette, add 1-2 mL of cabbage extract to a vial, and then add 5-7 mL of alginate solution (this ratio can be adjusted if desired, but no less than 1:1).
- Cap the vial and shake until well mixed.
- Using a clean transfer pipette, drop small amounts of the alginate+pigment solution into the calcium chloride solution (or calcium lactose) (**Fig 3**)

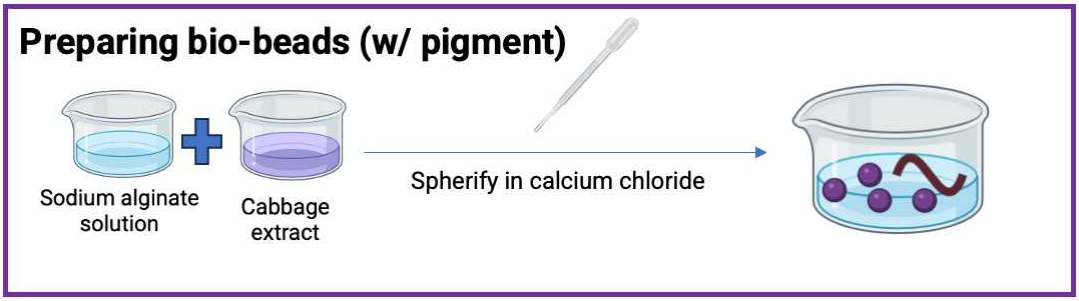

Figure 3. Cross link sodium alginate+anthocyanin mixture with calcium chloride (or calcium lactose). Spherification may be performed by dropping the mixture using a transfer pipette, but many other shapes are possible.

- Adjust the height of the pipette tip to alter bio-bead shape
- After at least 1 min, use the plastic spoon to remove bio-beads from the calcium solution.
- Place beads in plastic weigh boats

Note

A simple experiment to demonstrate cross-linking is as follows:
- Reverse the process in Figure 3, dropping the divalent cation (calcium) into the alginate mixture
- Observe any structures formed
- Compare the result to the spherification in **Fig 3**

6 *Step 6) pH Testing* 30m

- Using the white vinegar or bicarbonate solution and a clean transfer pipette, expose add a drop of solution (**Fig 4**)
- Wait approximately 1 minute.

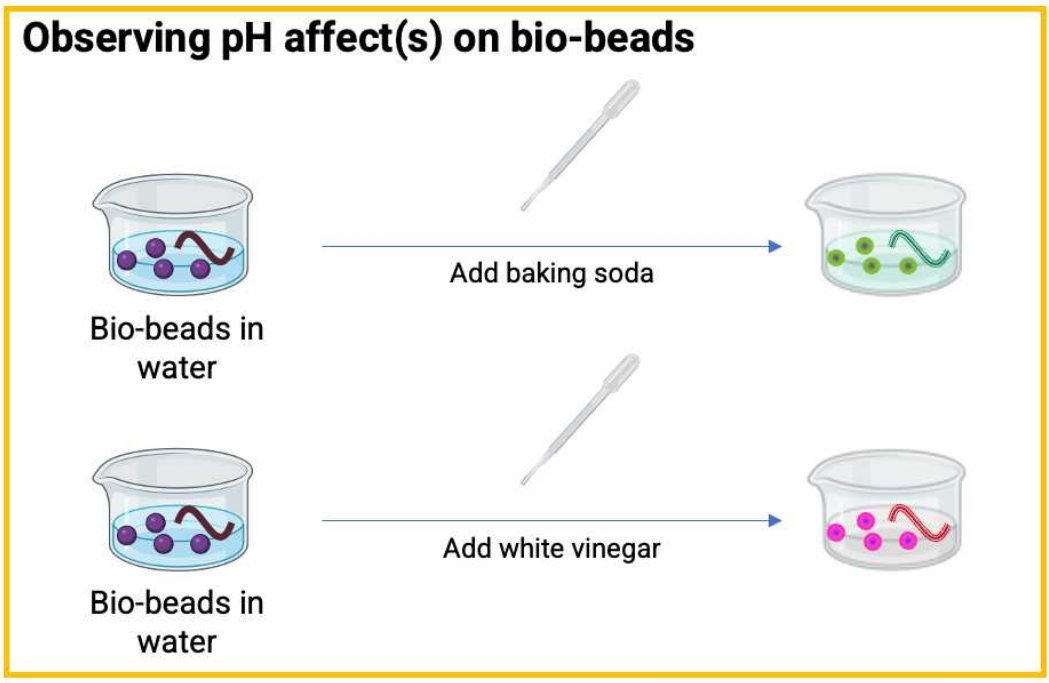

Figure 4. Test the change in pigment color after exposing beads to various solutions with known pH. (top) pH above 9 should produce a green color (approximately 520-560 nm). (bottom) pH below 5 should produce a pink/red color (approximately 680-720 nm).

- Observe any color changes and record notes in laboratory handout or notebook provided. Participants should connect the color observed with the wavelength of visible light according to the electromagnetic spectrum (**Fig 5**)

Expected result

- A solution pH above 9 should produce a green color (approximately 520-560 nm).
- A solution pH below 5 should produce a pink/red color (approximately 680-720 nm).

- If preferred, a cell phone app may be used to detect the color of the sample. For example, Color Name AR is a useful tool that is available for both iPhone and Android (as well as tablets).

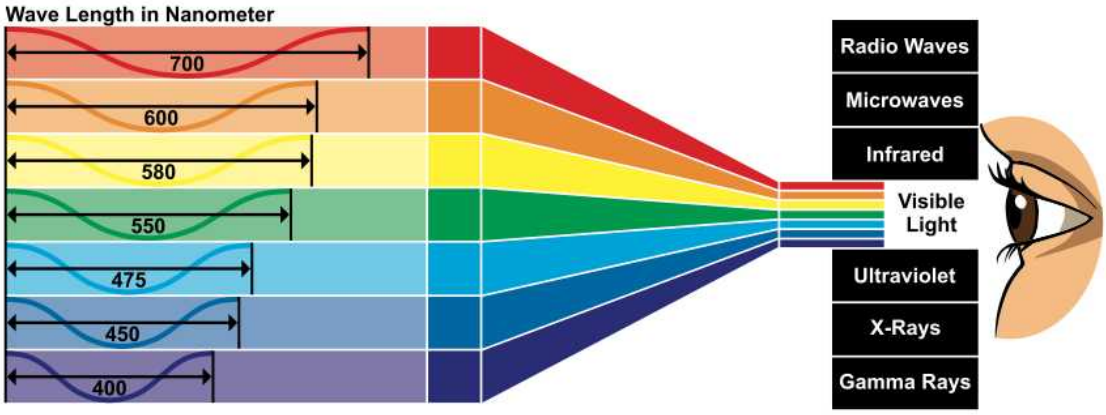

Figure 5. If visual observation of color change is not possible, a colorimeter or other device may be used to visually inspect the samples (see note below for example of mobile phone app). Image courtesy of ShutterStock standard license (no. 514067857).

Note

Link to ColorName AR:
https://apps.apple.com/us/app/color-name-ar/id906955675

- Participants should attempt to reverse any color change(s) back and forth, if possible.
- See Box for optional ideas that have been successful in various camp activities.

Note

ALTERNATIVE OPTION #1

Create bio-paper

- *Immerse a coffee filter (or other paper source) into the cryo-concentrate.*
- *Allow to dry, or use a blow dryer to expedite.*
- *Cut the bio-paper into strips*
- *Multiple layers of pigment may be used to enhace the color effect, if desired*
- *The bio-paper may be stored in a sealed container, out of direct sunlight, for up to 2 months.*

Note

ALTERNATIVE OPTION #2

Create bio-templated canvas for painting

- *Obtain a canvas and place on a flat, dry surface*
- *Place stencils on top of the canvas and have one team member hold firmly in place (or use a clamp)*
- *For best results, mix the cryo-concentrated extract with a gel material (e.g., crystalline nanocellulose, gelatin, Pluronic F-127, etc).*
- *Cast the gel+anthocyanin material in the stencil and then dry.*
- *Remove the stencil and you will have a pH-sensitive canvas and can apply solutions of varying pH to observe dynamic color changes in a 2D surface*

7 *Step 7) Clean Up* 15m

- Clean workstation/benchtop.
- All powders, gels and liquid materials are food grade and may be either composted or disposed in the standard trash receptacle.
- Used 50mL Falcon tubes and transfer pipettes may be reused after rinsing in warm water with soap.

Protocol references

1. Ocsoy, I. *et al.* Green synthesis with incorporated hydrothermal approaches for silver nanoparticles formation and enhanced antimicrobial activity against bacterial and fungal pathogens. *J Mol Liq***238**, (2017).
2. Demirbas, A. *et al.* Cryoconcentration of flavonoid extract for enhanced biophotovoltaics and pH sensitive thin films. *Biotechnol Prog* **34**, 206–217 (2018).
3. Demirbas, A. *et al.* Cryoconcentration of flavonoid extract for enhanced biophotovoltaics and pH sensitive thin films. *Biotechnol Prog* **34**, 206–217 (2018).