Natural pH Indicators and their Sensitivities to pH

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Abstract

Anthocyanins are a class of colorful compounds typically found in plant foods and are widely used for their color in the food industry. They tend to appear red, purple, blue, or black. Given anthocyanins' ability to fluctuate in their color properties, this instability has also been applied for use as a pH indicator in the food industry. This study analyzes the sensitivities of various anthocyanin-containing foods as pH indicators, as assessed by their color changing property and deviance in color between solutions with a pH difference of 0.5. Solutions of blackberries, black grapes, raspberries, blueberries, red cabbage and plums were derived, and reacted with household low-pigmented test ingredients ranging from a pH of 2 to 9. Pigmented aqueous solutions of red cabbage, blackberries, blueberries, plums, black grapes and raspberries showed a significant color difference between all pairs of test ingredients with a pH difference of 0.5.

Introduction

Anthocyanins are flavonoids, a class of hydroxylated (contains hydroxyl -OH group), polyphenolic (contains multiple phenol rings) compounds naturally found in plant foods, including common fruits, vegetables and grains (Fig. 1) (Hooper et al., 2008).

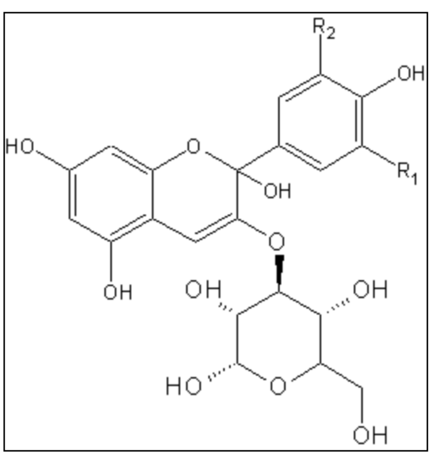

Fig 1. General Structure of Anthocyanins (component which undergoes reduction-oxidation reaction to act as pH indicator in various foods, such as red cabbage).

Anthocyanins are the main component in red cabbage that contribute to its ability to change colour depending on the pH of the substance it interacts with (Wiczkowski et al., 2013). Besides red cabbage, other produce which contains anthocyanins includes blueberries, raspberries, blackberries, grapes and plums (Lienard, n.d.). Since the listed foods vary in depth and intensity of their pigmentation, they likely vary in their concentration of anthocyanin.

Flavonoids are prone to different redox reactions depending on solution pH (Ibrahim et al., 2011a). At a low pH of 3, anthocyanins are present predominantly in flavylium cation form (structure shown in Figure 2 (C). Flavylium cation contributes to purple, orange, and red colours. At higher pH, kinetic and thermodynamic competitive effects are present as a hydration reaction (combining with water molecules) of flavylium cation and proton transfer reaction in anthocyanin's acidic hydroxyl groups. At pH 4, a violet quinoidal base is formed, which can undergo further deprotonation at pH between 6-7 to form blue toned quinonoid anions. However, at pH 5, anthocyanins instead take the form of a colorless carbinol pseudo-base structure. This can undergo further ring opening at pH 6, forming yellow retro-chalcones. Beyond pH 7, anthocyanins are degraded based on its substituent groups as it is less stable at high pH (Wahyuningsih et al., 2017).

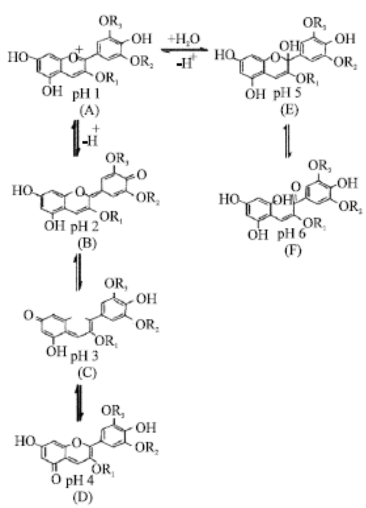

Fig 2. Anthocyanin Chemical Forms at Different pH Levels (from Ibrahim et al., 2011)

After completing the pH indicator lab using red cabbage, our group became curious as to whether other anthocyanin producing foods could also be used as effective pH indicators, in addition to red cabbage which has been deemed a suitable natural pH indicator (Chigurupati et al., 2002). We chose to carry out this experiment for our term project to determine whether these anthocyanin-producing foods could potentially be used as natural, food safe, affordable, yet effective alternatives to traditional pH tests and indicators. Given the importance of pH in industry, such as the food industry, this could be a quick, easy, and affordable pH test for e.g. small restaurant businesses. Depending on the results, we could infer which foods have potential as accessible, cost-effective pH indicators.

Thus, the aim of this study is to investigate how anthocyanin containing foods that vary in depth and shade behave when exposed to acid and basic household aqueous solutions of various pH levels (pH 2-9), and if they are sensitive to pH differences of 0.5. Although we initially started with seven foods, only six were included in the analysis of this experiment upon discovering that beetroot contained another compound which may alter results (Eshaghi et al., 2020). The primary research question for this study is then as follows: Which of the following anthocyanin-containing natural food items (Blackberries, Black grapes, Raspberries, Blueberries, Red cabbage and Plums) are sensitive to pH differences of 0.5?

Methods

First, the collection of 45 plastic cups, a coffee filter and holder, water kettle, pot, cutting board, knife, teaspoon, masking tape and a permanent marker was completed (Fig. 3). To make the aqueous pigmented solution to test out this experiment, half a red cabbage was roughly chopped and placed into a pot. The pot was fully saturated with boiling water and left untouched for 30 minutes. Then, ¼ cup of six clear, low-pigmented ingredients that have known pH values (Baking soda, Egg white, Homogenized Milk, Evian water, Lemon juice, and Vinegar) were collected while waiting for the boiled water to fully saturate the red cabbage. After 30 minutes, the red cabbage was strained out and the pigmented solution was saved. Once the pigmented solution had cooled, ⅓ cup of the solution was poured through a filter into the seven clear plastic cups. One teaspoon of each collected ingredient was added to a different cup that contained the pigmented solution. Please note that every cup was labeled, and one cup did not have any additional ingredient added to it; this served as the control. The baking soda and egg whites' solutions, milk and water solutions, and lemon juice and vinegar solutions were then compared to observe whether any differences in color were visible. any visible difference in color was compared. Observations and results were diligently recorded into the lab notebook.

Fig 3. Apparatus that was used to carry out this experiment (plastic cups (45), coffee filter and holder, water kettle, pot, cutting board, knife, teaspoon, masking tape, permanent marker).

Fig 4. 6 different anthocyanin-containing food items prepared to test which would be the best pH indicator. From left: ½ of red cabbage, 2 cups of blackberries, 3 plums, 2 cups of black grapes, 2 cups of blueberries, 2 cups of raspberries, were used for this experiment.

The above steps were repeated for each of the different anthocyanin-containing food items, blackberries (2 cups), blueberries (2 cups), raspberries (2 cups) , plums (3) and black grapes (2 cups) (Fig. 4). Note that each food item yielded a different colored pigmented solution.

After the experiment was completed, the results derived from all the food items were compared to analyze which were sensitive to a pH difference of 0.5, and which were not, based on if a colour difference was observed upon addition of ingredients differing in pH by 0.5 from one another.

Results

Through this experiment, it was found that each natural food produced a varying range of colours along the pH continuum (Fig. 3). Observational analysis allowed for the derivation that all of Red Cabbage, Blackberries, Blueberries, Plums, Black Grapes and Raspberries were sensitive to a pH difference of 0.5, and displayed different colours between the lemon juice and vinegar solutions, homogenized milk and Evian water solutions, and baking soda and egg white solutions (Fig. 3). Red cabbage displayed the most distinct colour difference across the pH spectrum (Fig. 3).

Another prominent finding was that for Blackberries, Blueberries, Plums, Black Grapes and Raspberries, a more noticeable colour difference was seen between the baking soda and egg white solutions as compared to lemon juice and vinegar solutions. Also, upon addition of homogenized milk to the pigmented solutions of these five foods, white particle formation was observed. Moreover, the addition of baking soda to these five foods resulted in evident bubble/froth formation at the surface of the liquid.

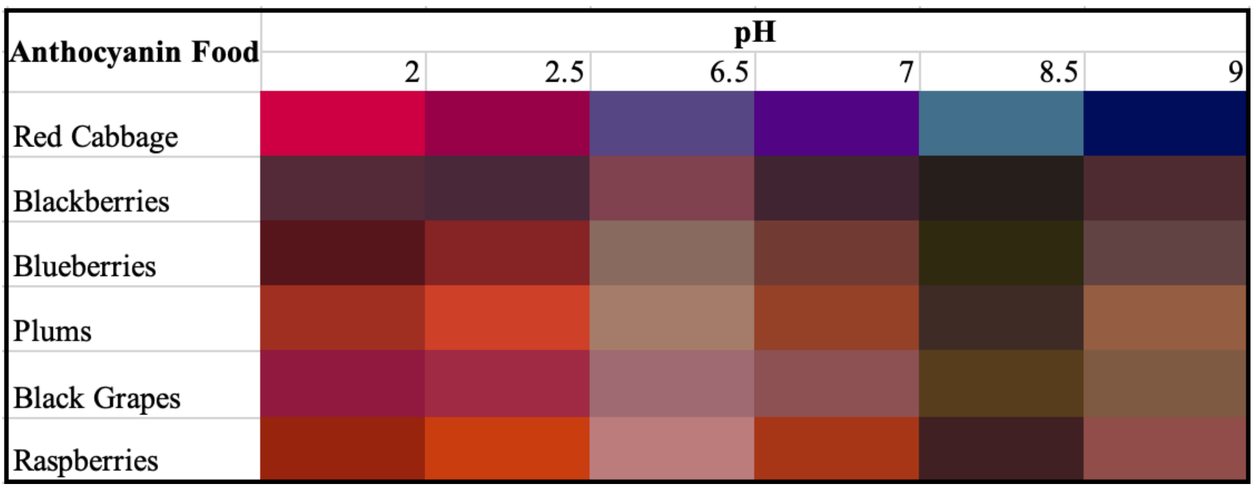

Fig 5. Visual portrayal of the multitude of colours observed for each food's pigmented solution upon addition of ingredients with varying pH. Lemon juice represents pH=2, vinegar is pH=2.5, homogenized milk is pH=6.5, Evian water is pH=7, baking soda is pH=8.5, and egg white is pH=9. Figure generated on Microsoft Excel.

Discussion

The objective of this study was to analyze sensitivities of various anthocyanin-containing foods to pH and to draw insight into their potential as natural pH indicators. The magnitude to which the solution deviated following each successive change in pH of 0.5 was used as a criterion to differentiate between successful indicators. Interestingly, all tested anthocyanin containing foods displayed sensitives to pH difference of 0.5 across the pH scale (pH=2 and pH=2.5, pH=6.5 and pH=7, pH=8.5 and pH=9). From this result, it is inferred that each of the foods tested (red cabbage, blueberries, blackberries, plums, black grapes, and raspberries) have the potential to be effective natural pH indicators.

An intriguing finding was that although most anthocyanin-containing foods showed a color difference between the lemon juice and vinegar solutions, this difference was less pronounced and easily discernible as compared to the color deviance between the baking soda and egg whites solutions (Khoo et al., 2017). A potential reason for this could be that since most of the foods analyzed are acidic themselves (blackberries, blueberries, plums, black grapes, raspberries), they did not show a great deviance of color upon addition of solutions with varying extents of acidity. In contrast, there was a distinct dissimilarity in color between solutions of varying basicity, and a potential reasoning for this could be that the extent of acid-base reactions with the pH= 8.5 solution and pH=9 solution =differed significantly. In parallel, red cabbage has a pH equaling to approximately 7, which is likely why it showed a pronounced difference between pH=2.5 and pH=3 as well as pH=6.5 and pH=7 (Ibrahim et al., 2011).

White particle formation was observed with the addition of homogenized milk in various pigmented solutions. This may be due to acid coagulation of the milk resulting in curdling (Lucey & Singh, 2003). Milk contains proteins called casein which are amphiphilic (containing hydrophobic and hydrophobic regions) and form micelles. When acid is added to the milk, this introduces positive ions which essentially attract the negative micelles and neutralizes them (Lucey & Singh, 2003). Consequently, casein proteins are rendered denatured and are unable to interact with one another as previously, which causes the clumping of proteins. Blackberries, blueberries, black grapes, plums, and raspberries are all slightly acidic foods, which could account for the curdling of the milk and white particle formation seen in these foods' pigmented solutions.

Additionally, slightly acidic foods (blackberries, blueberries, plums, black grapes and raspberries) when combined with baking soda in their pigmented solutions produced froth/bubbles. When baking soda is mixed with an acid, a reaction ensues in which one of the products is carbon dioxide gas (Baltrusaitis et al., 2007). It is inferred that the froth/bubble formation seen in the pigmented solutions of these foods upon addition of baking soda is due to carbon dioxide gas formation (gas bubbles). Also, the froth/bubble formation disappeared after approximately 2-3 minutes. This could be due to the gas bubbles breaking the surface of the liquid and effectively escaping into the air (Baltrusaitis et al., 2007).

One of the limitations of this study include observer bias or the tendency to perceive expected results as observed ones. Also, the detection of color is subjective; it often depends on how the observer perceives the visual representation (Shevell, 2003). The context in which an object is presented influences the perception of color, therefore different observers yield the identification of different colors (Shevell, 2003). A way to overcome this would be to have other group members or peers conduct the experiment as well, to minimize observer bias. Observations could then be pooled to analyze consistencies or inconsistencies in colors seen and draw final results. Additionally, increasing the number of replicates in the sample within this study will also provide a more detailed picture of which foods were the most vulnerable to pH changes.

Results from this experiment provide insight into other pigmented foods which can potentially be used as natural pH indicators, serving as sustainable alternatives to generally costly lab-based pH indicators. Also, alternative practical applications of pH indicators derived from natural food have been explored as well. For example, in fish products, pH indicators are used to determine whether a food is safe to consume. A rise in pH of the fish product is directly correlated with spoilage; this property can be utilized to create anthocyanin-based pH indicators to monitor food safety risks. Shi et al. (2020) extracted anthocyanins from blueberry peels to assess the freshness of tilapia. Two film wraps were developed from the extraction of anthocyanins to function as colorimetric sensors. The authors predicted that any changes to pH would be detected by the films providing a clear indication of safe/unsafe food consumption. It was concluded that the films were sensitive to fish pH change and could act as food safety labels. This result may help create natural, low-cost methods to control food quality (Shi et al., 2021). Subsequently, the results from our experiment shed light on other foods which could be utilized for this purpose.

An extension of this experiment could be conducted by testing color change/difference upon the addition of ingredients with pHs over the rest of the spectrum to see if similar sensitivities to pH are observed. Furthermore, other anthocyanin-containing foods could be included as well, such as pomegranate, blue corn and concord grapes, to analyze the results of these foods and their potential as pH indicators.

Conclusion

The purpose of this study was to analyze the sensitivities of various anthocyanin-containing foods (red cabbage, blackberries, blueberries, plums, black grapes and raspberries) to pH differences of 0.5, based on observed color deviance upon addition of varying ingredients with such pH variance. The results derived demonstrate that red cabbage, blackberries, blueberries, plums, black grapes and raspberries display such sensitivity across varying points of the pH spectrum analyzed. These results provide valuable insight into the potential of these various anthocyanin-containing foods as sustainable alternatives to commonly used lab-based pH indicators.

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Appendix

Table 1. Initial colors observed (control) of pigment solutions derived from each of the anthocyanin-containing foods (Red Cabbage, Blackberries, Blueberries, Plums, Black Grapes, Raspberries).

Food	**Solution Colour**
Red cabbage	Bright, opaque purple-blue
Blackberries	Deep, slightly translucent red-purple
Blueberries	Dark, translucent dull orange
Plums	Bright, translucent pink-orange
Black Grapes	Dull, slightly translucent pink
Raspberries	Bright, translucent red

Table 2. Initial colors and properties observed for the test ingredients prior to addition to the pigmented solutions (lemon juice, vinegar, homogenized milk, Evian water, baking soda and egg white).

Test Ingredient	**Colour**	**Other Properties**
Lemon Juice	Light white tint	Liquid
Vinegar	Clear	Liquid
Homogenized Milk	Light, white	Liquid
Evian Water	Clear	Liquid
Baking Soda	White	Powder
Egg White	Light white-yellow	Viscous liquid

Table 3. Observations of color change to the Red Cabbage, Blackberries, Blueberries, Plums, Black Grapes, and Raspberries pigmented solutions upon addition of test ingredients (lemon juice, vinegar, homogenized milk, Evian water, baking soda and egg white).

Anthocyanin-containing food	**Test Ingredient (T.I.)**	**Initial pH of T.I.**	**Observations**
Red cabbage	Lemon Juice	2	Changed to bright pink, more opaque
	Vinegar	2.5	Changed to magenta
	Milk	6.5	Pale purple-blue, cloudy
	Evian water	7	No colour change

	Baking soda	8.5	Dark teal-blue
	Egg white	9	Dark blue
Blackberries	Lemon Juice	2	Slightly more red, a little cloudy
	Vinegar	2.5	Slightly more red
	Milk	6.5	Dull lavender purple
	Evian water	7	No colour change
	Baking soda	8.5	Darker, almost black, slight foam at top
	Egg white	9	Slightly more brown tone
Blueberries	Lemon Juice	2	Darker, more purple-red
	Vinegar	2.5	More orange and brighter
	Milk	6.5	Changed to light grey-brown
	Evian water	7	Slightly lighter - same colour tone
	Baking soda	8.5	Darker brown-yellow, slight froth at top
	Egg white	9	Paler and darker, purple-brown
Plums	Lemon Juice	2	More opaque, reddish dull colour
	Vinegar	2.5	Slightly brighter
	Milk	6.5	Extremely light pale pink, white particles throughout
	Evian water	7	Slightly lighter
	Baking soda	8.5	Froth at top, dark brown with yellow tint
	Egg white	9	Paler, lighter, beige

Black grapes	Lemon Juice	2	Changed to darker, brighter pink (magenta)
	Vinegar	2.5	Darker, brighter pink
	Milk	6.5	Light Grey-purple, with white particles
	Evian water	7	Slightly paler colour
	Baking soda	8.5	Pale dark green, but translucent
	Egg white	9	Yellow-green, gray (dull beige)
Raspberries	Lemon Juice	2	More opaque, more darker colour
	Vinegar	2.5	Slightly brighter
	Milk	6.5	Light pink, opaque, white particles, cloudy
	Evian water	7	No colour change
	Baking soda	8.5	Darker purple/cola colour, froth at top
	Egg white	9	Changed to pink

Table 4. Observations of whether or not a colour difference was seen between solutions with a pH difference of 0.5 (lemon juice: pH=2, vinegar: pH=2.5, homogenized milk: pH=6.5, Evian water: pH=7, baking soda: pH=8.5, egg white: pH=9) for each anthocyanin-containing food analyzed.

Food	Solutions Compared	pH difference	Colour Difference
Red cabbage	Lemon juice and Vinegar	0.5	Yes
	Homo. milk and Evian water	0.5	Yes
	Baking soda and Egg White	0.5	Yes
Blackberry	Lemon juice and Vinegar	0.5	Yes
	Homo. milk and Evian water	0.5	Yes
	Baking soda and Egg White	0.5	Yes

Blueberries	Lemon juice and Vinegar	0.5	Yes
	Homo. milk and Evian water	0.5	Yes
	Baking soda and Egg White	0.5	Yes
Plum	Lemon juice and Vinegar	0.5	Yes
	Homo. milk and Evian water	0.5	Yes
	Baking soda and Egg White	0.5	Yes
Black grapes	Lemon juice and Vinegar	0.5	Yes
	Homo. milk and Evian water	0.5	Yes
	Baking soda and Egg White	0.5	Yes
Raspberries	Lemon juice and Vinegar	0.5	Yes
	Homo. milk and Evian water	0.5	Yes
	Baking soda and Egg White	0.5	Yes

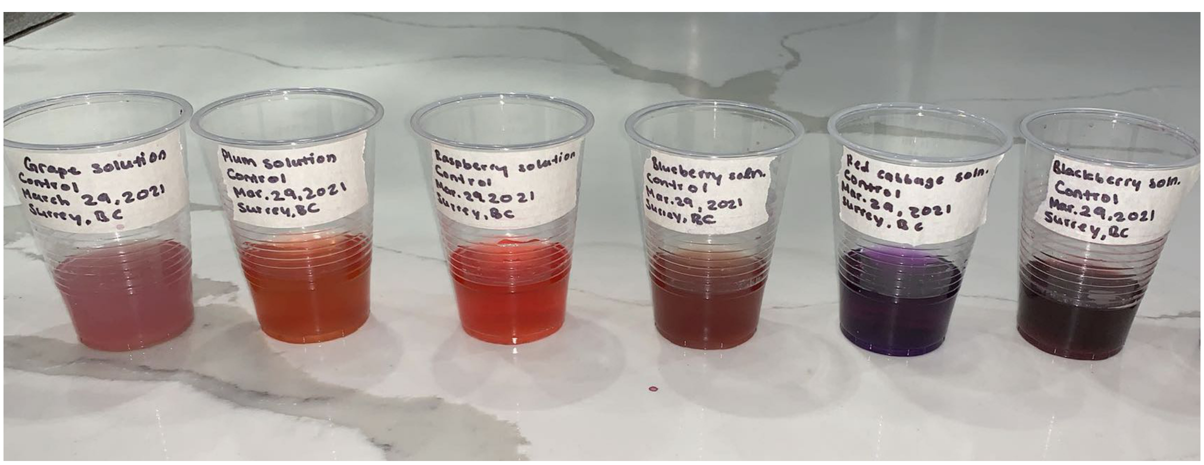

Fig 6. Photograph of pigmented control solutions (no additional ingredient added) of each food analyzed (Red Cabbage, Blackberries, Blueberries, Plums, Black Grapes, and Raspberries).

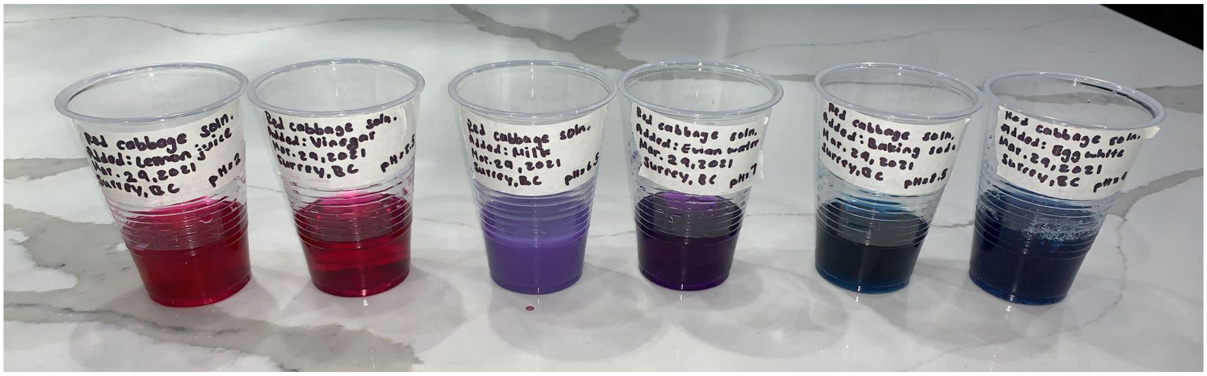

Fig 7. Photograph of observed colours upon addition of test ingredients (lemon juice: pH=2, vinegar: pH=2.5, homogenized milk: pH=6.5, Evian water: pH=7, baking soda: pH=8.5, egg white: pH=9) to Red Cabbage pigmented solution.

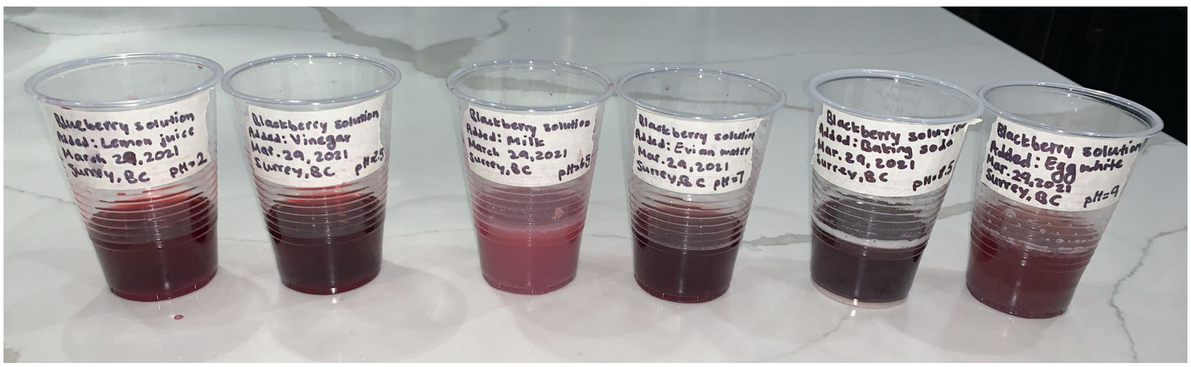

Fig 8. Photograph of observed colours upon addition of test ingredients (lemon juice: pH=2, vinegar: pH=2.5, homogenized milk: pH=6.5, Evian water: pH=7, baking soda: pH=8.5, egg white: pH=9) to Blackberry pigmented solution.

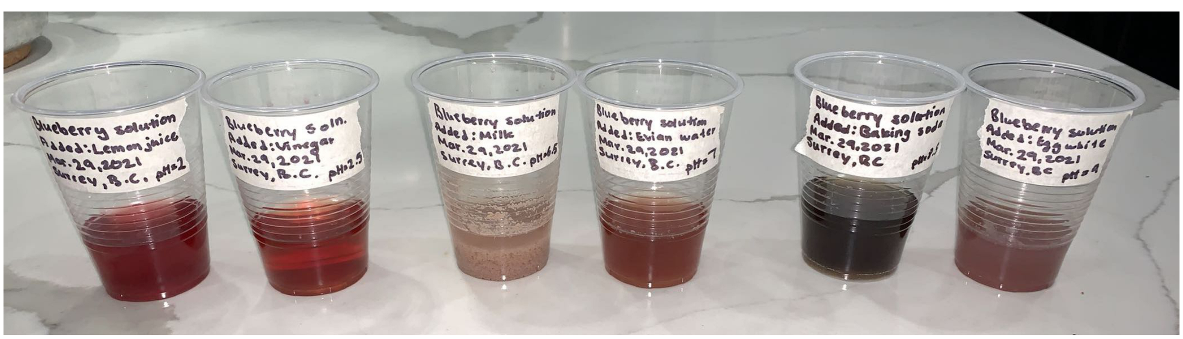

Fig 9. Photograph of observed colours upon addition of test ingredients (lemon juice: pH=2, vinegar: pH=2.5, homogenized milk: pH=6.5, Evian water: pH=7, baking soda: pH=8.5, egg white: pH=9) to Blueberry pigmented solution.

Fig 10. Photograph of observed colours upon addition of test ingredients (lemon juice: pH=2, vinegar: pH=2.5, homogenized milk: pH=6.5, Evian water: pH=7, baking soda: pH=8.5, egg white: pH=9) to Plum pigmented solution.

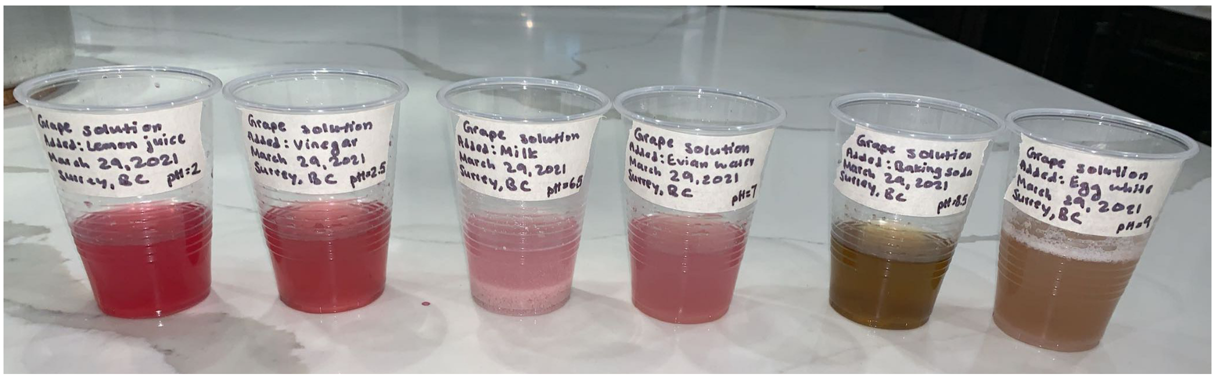

Fig 11. Photograph of observed colours upon addition of test ingredients (lemon juice: pH=2, vinegar: pH=2.5, homogenized milk: pH=6.5, Evian water: pH=7, baking soda: pH=8.5, egg white: pH=9) to Black Grape pigmented solution.

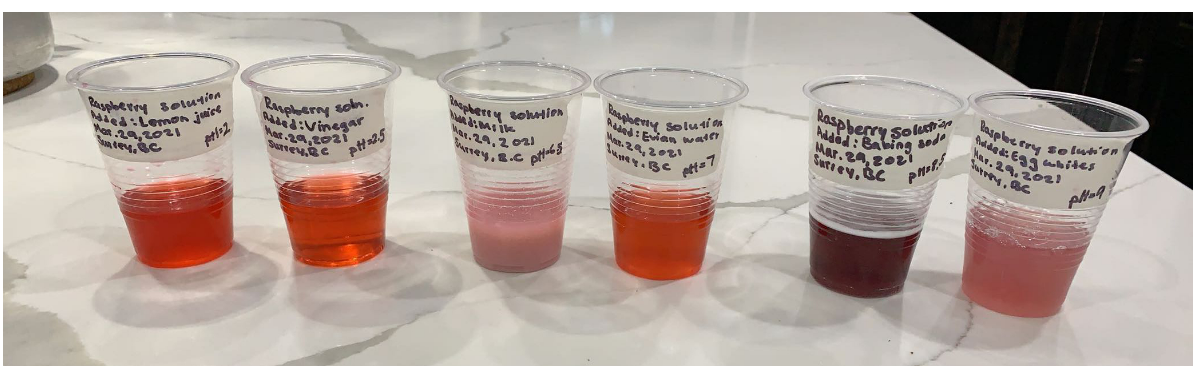

Fig 12. Photograph of observed colours upon addition of test ingredients (lemon juice: pH=2, vinegar: pH=2.5, homogenized milk: pH=6.5, Evian water: pH=7, baking soda: pH=8.5, egg white: pH=9) to Raspberry pigmented solution.