Plant Pigments Lab Report

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Section A07 wed 5-8

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I. PURPOSE/OBJECTIVE:

The purpose of this experiment is to see how pH levels of certain vegetables (cauliflower, cabbage, carrot and broccoli) change while being cooked and how the varying pH levels change the coloring of the vegetables. This experiment is based on different pigments in vegetables. We will see the effects of acidic (vinegar), basic (baking powder) and neutral (DI water) solution on these same pigments.

Pigment and any coloring matter in plant that reflects light of certain wavelengths. Based on our lectures: The major groups of plant pigment are the chlorophylls, carotenoids, anthocyanins, and flavonoids. Chlorophyll is a green pigment. Chlorophyll is a fat soluble plant pigment. Loss of Mg+2, the phytol group, and/or the methyl ester group as well as ring oxidation are all changes that affect the color of chlorophyll. The chlorophyll molecule is sensitive to heat and unstable in the presence of acid. Chlorophyll a is degraded faster than chlorophyll b under any given conditions. The chlorophyll molecule is most stable at neutral pH. In very acidic media the Mg+2 in chlorophyll is not only displaced, but the phytol groups are also lost resulting in the formation of pheophorbide; a dull olive-brown color and water soluble. In an alkaline environment both the phytol and methyl ester groups are lost resulting in the formation of chlorophyllin which is bright green in color and water soluble. The enzyme chlorophyllase catalyzes the hydrolysis of the phytol group off of the chlorophyll molecule resulting in the formation of chlorophyllide which has a green color and is water soluble. If chlorophyllide loses its Mg+2, pheophorbide is formed.

Cartenoids are the pigments responsible for yellow and orange color. The carotenoids are a group of lipid soluble pigments. Being highly unsaturated and lipid soluble, carotenoids are very sensitive to oxidation in the presence of oxygen, light, or acid. Carotenoids are more stable when associated with sugars, proteins, or fatty acids in vivo. When plant tissue is damaged and carotenoid complexes are destroyed, the carotenoid becomes more sensitive to oxidation. For example, in crustacean shells the carotenoid astaxanthin is combined with protein. Upon heating the astaxanthin-protein complex is broken apart and the shell turns from blue-grey to orange-red. The resulting free carotenoid is now more susceptible to oxidation. Anthocyanins are the third major class of plant pigments and they are water soluble pigments.

As the temperature rises, the pH will lower, and the lower the level of pH, or the higher the acidity, the more the pigment will lose its natural color. During the process of this experiment, we learned many things about the nature of both pH and pigment. Heat and pH separately, change the color (pigment) of the vegetables.

When we add solutions with different pHs to observe color changes; an alteration occurs. Changing the pH increases hydrogen ions, or protons, in the cell, and this result in the denaturalization of proteins, or the change of shape. The new hydrogen ions create a gradient which is different than before and the proteins will change accordingly. This changed amount of ions makes it more likely that the bonding of proteins will be interrupted and the protein will unfold and lose their structures. The pH solutions increased the amount of ions and the likelihood of protein denaturalization and a structure change. The denaturalization allowed the hydrogen ions to move around and this changed the shape of the protein and the acidity of the cell, which in turn changed the color of the vegetable. We saw that with the broccoli, the chlorophyll got brighter in response to the change in pH.

2- Spectrophotometers may be used to obtain the absorption spectrum of a compound or the absorbance at any given wavelength of light by a sample(FD&C blue #1, FD&C yellow #5, FD&C red #40, and green). It is important to specify the wavelength of light that an absorbance measurement is taken at because in most cases the absorption of light by a sample is strongly dependent on the wavelength of the light source. In this experiment, we measured and analyzed the visible light absorbance spectra of various samples of aqueous dye mixtures to determine the absorbance spectrum for each sample and Compare the spectra of various dye mixtures. Different dyes absorb at different wavelengths. We measured the absorbance of dyes, mixed with water, over the 400 – 700 nm (every 20 nm) range.

Light is composed of photons with wavelengths and energies. The longer the wavelength, the lower the energy. Spectrophotometry is the study of the transmission or absorbance of light through a substance. Transmittance is a measure of the amount of light passing through a substance; absorbance is the amount of light that was captured by a substance. A clear colorless piece of glass has close to 100% transmittance and 0 absorbance of visible light. In colored liquids, for example, the color we see is a result of the different wavelengths absorbed and total amount of light absorbed.

II. Data:

pH Values Before and After Cooking

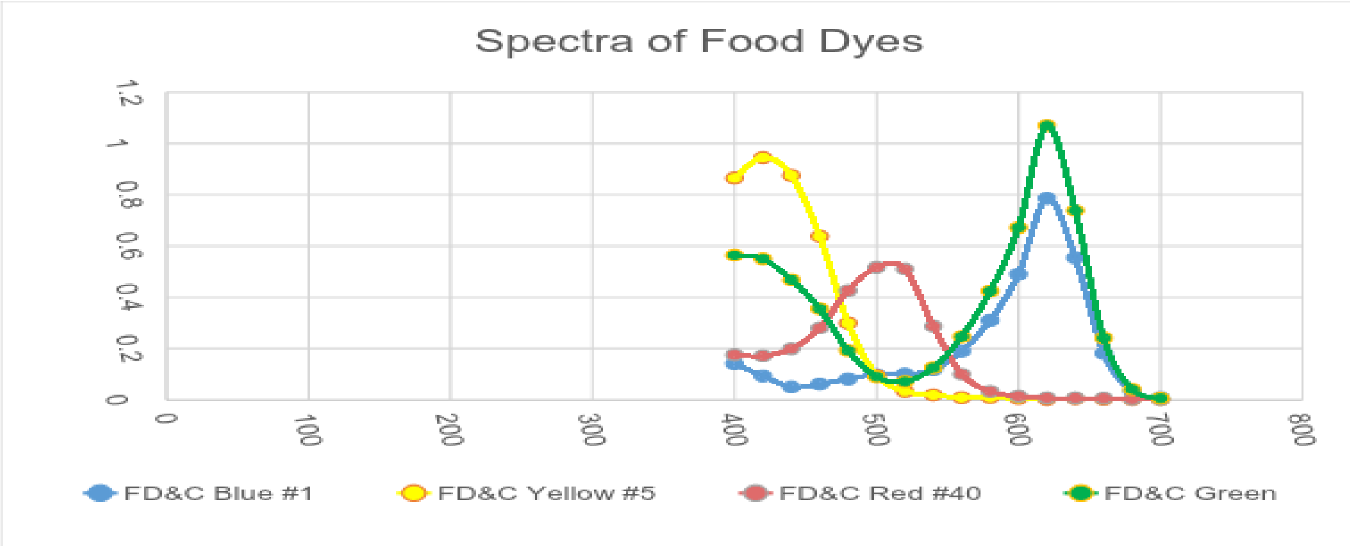
Group	Vegetables	Acid pHraw	Acid pHcook	Base pHraw	Base pHcook	Neutral pHraw	Neutral pHcook
A	Carrots	3.50	3.89	8.08	8.84	6.91	7.05
A	Broccoli	3.41	3.95	8.29	8.93	7.38	7.33
B	Carrots	2.62, 3.45	3.45	8.25	9.11	7.44	,7.95
B	Broccoli	2.69	3.21	8.61	8.91	6.69	7.18
C	Carrots	3.29	3.52	8.12	9.36	6.93	7.18
C	Broccoli	3.11	3.89	8.14	9.45	7.0	6.89
D	Red Cabbage	3.55	3.76	8.13	9.20	7.30	6.99
D	Cauliflower	3.38	3.80	8.l4	9.07	7.77	8.38
E	Red Cabbage	3.47	3.73	8.07	8.88	7.60	6.91
E	Cauliflower	3.30	3.88	8.0	8.84	7.2	7.2
F	Red Cabbage	3.48	3.88	8.13	9.38	7.29	6.97
F	Cauliflower	3.48	3.93	8.13	9.55	7.29	8.15

LAB Values

Group	Vegetables	Acid (order: LAB)	Base	Neutral
A	Carrots	L:46.01 A: 12.37 B: 14.07	L:44.41 A: 15.09 B: 15.89	L: 46.20 A: 14.97 B: 15.64
A	Broccoli	L: 45.97 A: -3.73	L: 44.11 A: -7.26	L: 43.11 A: -7.70

		B: 9.10	B: 7.99	B: 8.65
B	Carrots	L: 54.71 A: 16.32 B: 18.01	L: 44.11 A: 9.92 B: 13.33	L: 53.55 A: 17.11 B: 15.43
B	Broccoli	L: 47.76 A: -3.03 B: 9.91	L: 42.33 A: -7.99 B: 9.21	L: 48.33 A: -6.46 B: 8.06
C	Carrots	L: 55.11 A: 16.47 B: 17.89	L: 44.10 A: 9.83 B: 13.06	L: 53.65 A: 17.48 B: 15.23
C	Broccoli	L: 58.57 A: -4.88 B: 10.76	L: 40.47 A: 4.39 B: 5.59	L: 51.85 A: -9.00 B: 10.81
D	Red Cabbage	L: 37.70 A: 8.75 B: -2.91	L: 36.37 A: 6.13 B: -1.73	L: 37.02 A: 3.26 B: -3.78
D	Cauliflower	L: 63.98 A: -3.05 B: 8.75	L: 59.67 A: -4.93 B: 11.25	L: 62.32 A: -4.02 B: 9.57
E	Red Cabbage	L: 27.09 A: 13.35 B: -3.49	L: 35.86 A: 0.110 B: -2.16	L: 37.54 A: 2.85 B: -3.57
E	Cauliflower	L: 64.00 A: -3.25 B: 8.45	L: 55.76 A: -4.63 B: 11.28	L: 56.77 A: -3.04 B: 5.82
F	Red Cabbage	L: 27.09 A: 13.35 B: -3.49	L: 35.86 A: 0.110 B: -2.16	L: 37.54 A: 2.85 B: -3.57
F	Cauliflower	L: 69.81 A: -2.89 B: 9.81	L: 53.41 A: -2.38 B: 10.70	L: 67.17 A: -4.74 B: 8.66

Spectra of Food Dyes and Munsell Values



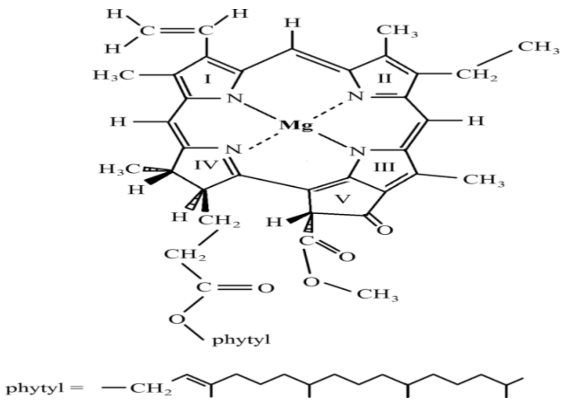
Munsell Readings:

Group	Vegetables	Acid	Base	Neutral
A	Carrots	2.5YR, 6/12	2.5 YR,6/16	2.5YR, 7/12
A	Broccoli	7.5GY, 6/8	5GY, 5/10	5GY, 6/10
B	Carrots	2.5YR, 5/14	2.5YR, 7/14	2.5YR, 6/12
B	Broccoli	2.5GY, 7/6	2.5GY, 7/10	2.5GY, 6/8
C	Carrots	2.5YR, 6/12	2.5YR, 5/14	2.5YR, 6/16
C	Broccoli	2.5 GY, 9/4	5GY, 7/8	5GY, 5/8
D	Red Cabbage	10P, 4/10	10G, 2/4	5P, 2/6
D	Cauliflower	2.5GY, 9/2	2.5GY, 9/4	5GY, 9/2
E	Red Cabbage	2.5RP, 3/10	7.5BG, 3/8	5P, 3/8
E	Cauliflower	10Y, 9/2	5 GY, 9/6	2.5GY, 9/2
F	Red Cabbage	2.5RP, 4/12	5G, 3/8	10PB, 4/4

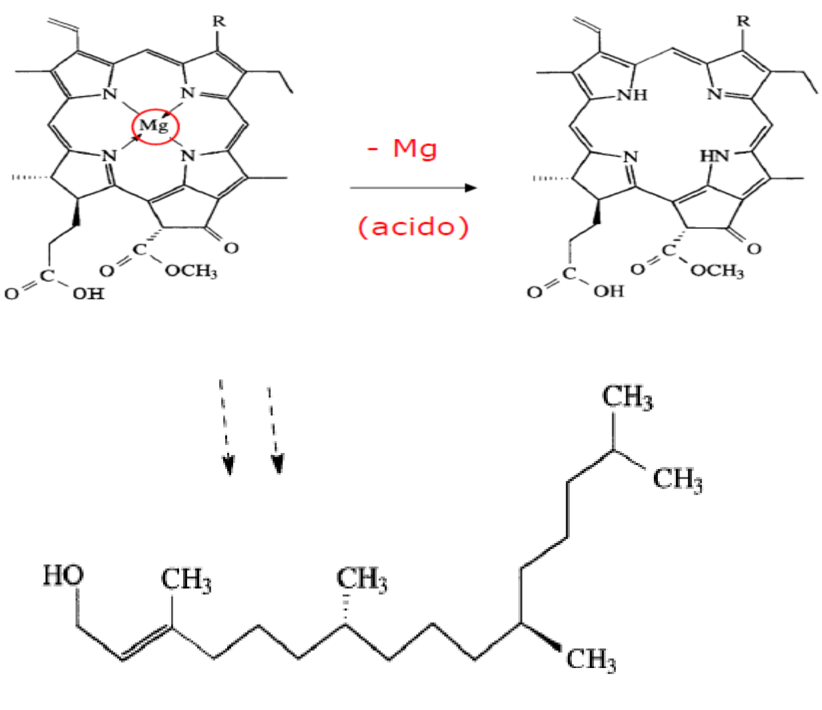
F	Cauliflower	10Y, 9/2	4GY, 9/6	2.5GY, 9/2

III Questions:

1- Chlorophyll is green at neutral pH. Hydro-solubile. Chlorophyll is brown at acidic ph (loss of Mg)

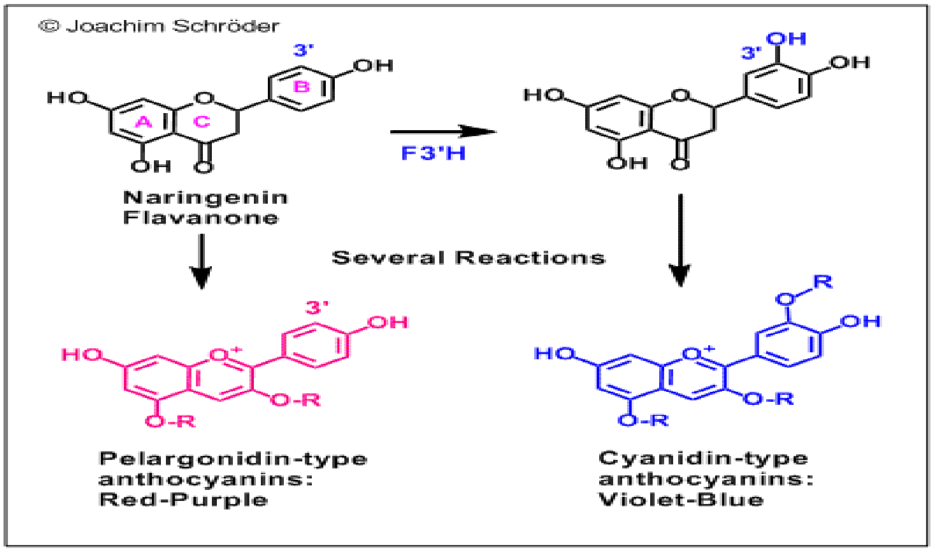


Chlorophyll



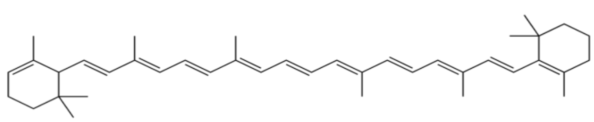
Phytol

Anthoxanthins

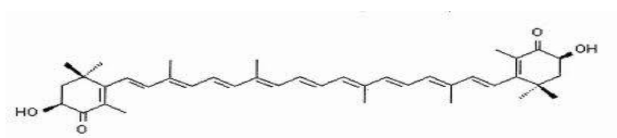


Flavylium cation I: pH < 1, red
Chromenol II: pH = 4-5, colorless
Quinoidal base III: pH = 6-7, purple
Chalcone V: pH = 7-8, yellow
Ionic anhydro base IV: pH = 7-8, deep blue

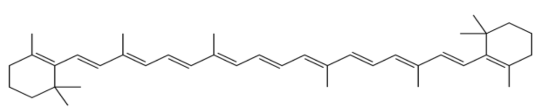
Alpha-carotene



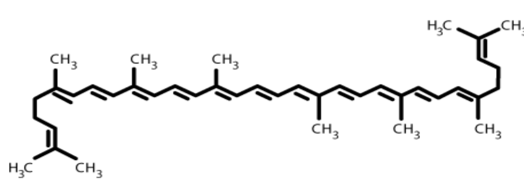
Astaxanthin



Beta-carotene



Lycop



2- Carrots have carotenoid, beta-carotene (deep orange/yellow) source, and cauliflower has anthoxanthins (white) for plant pigmentation, a flavonoid source. In an acidic environment the color of flavonoids may be white or ivory. In alkaline media the color is intensified. If there are > 2 unsubstituted hydroxyl groups can chelate metal ions such as iron or aluminum and to act as potential substrates in enzymatic browning reactions in plants. Flavonoids are more stable to heat and oxidation than anthocyanins. Anthoxanthins are water soluble. Cauliflower pH results via cooking had slightly different results. In some groups pH values changed where the acidity decreased, the basicity increased, and the neutral solution turned slightly basic. In water related cooking, such as boiling, there is a less temperature change as the vegetables are exposed to the heat. Temperature stays the same, and does not lose heat it takes less time to cook. The enzymes that work on the alteration of pigments have less time to work on the pigments which results in less color change. In the boiling the least change was noticed.

Carotenoids are fat soluble pigment and they are generally stable to ordinary methods of cookery. Being highly unsaturated and lipid soluble, carotenoids are very sensitive to oxidation in the presence of oxygen, light, or acid. Carotenoids are more stable when associated with sugars, proteins, or fatty acids in vivo. When plant tissue is damaged and carotenoid complexes are destroyed, the carotenoid becomes more sensitive to oxidation. For example, in crustacean shells the carotenoid astaxanthin is combined with protein. Upon heating the astaxanthin-protein complex is broken apart and the shell turns from blue-grey to orange-red. The resulting free carotenoid is now more susceptible to oxidation. In testing carrot pH was affected in a way where all the acid treated, base treated, and neutral solution pH's increased. The beta-carotene in carrots was not affected heavily by pH changes.

3. Anthoxanthins are formed in ring with OH groups and ketone groups attached to the rings they are water soluble and they can easily get oxidized and lose their colors with pH and heat changes. In an acidic environment the color of flavonoids may be white or ivory. In alkaline media the color is intensified. If there are > 2 unsubstituted hydroxyl groups can chelate metal ions such as iron or aluminum and to act as potential substrates in enzymatic browning reactions in plants. Flavonoids are more stable to heat and oxidation than anthocyanins. Anthoxanthins are water soluble. As it was said before carotenoids are fat soluble pigments and they are highly unsaturated, this means that they have a lot of double bonds in their structures. Because of their molecular stability these pigments are not affected heavily through pH changes or heat stress but because of these double bonds it is easier to become oxidized by oxygen and they can lose their color.

4. munsell color system is related to the visual spectrum. Value dimension is where lightness or darkness is varied with a given hue; chroma is the saturation that is related but distinct concepts referring to the perceived intensity of a specific color. Chroma is the colorfulness relative to the brightness of another color that appears white under similar viewing conditions. Saturation is the colorfulness of a color relative to its own brightness, and what the values of Munsell color system are given for different colors and Hue that shows the actual color on the visible spectrum on the Munsell Hue Circle.