How does pH affect the redness of fruits that contain anthocyanin pigments?

Year 12

Introduction

A fruit's colour is a critical factor in consumer attraction and purchase decision. Colour is widely known by customers that it provides an indication of a fruit's freshness, ripeness and flavour quality. Discolouration or colours that are not appropriate for the product is a quality defect in fruit and low intensity of red colour indicates lack of ripeness (Barrett et al., 2010) for red fruits. Therefore, red fruits with high intensity of redness and lack of discolouration is favoured and attractive for consumers.

pH has a great impact on fruit pigments such as anthocyanin which determines the colour of fruit such as strawberries and red grapes. At unfavoured pH, red fruits may become unattractive because pH changes the intensity of the red pigment (Andrés-Bello, et al., 2013). Thus, the knowledge in the effect of pH on fruit pigments is necessary to produce attractive red fruits.

Aim and Hypothesis

The aim of this depth study is to investigate how pH affects anthocyanin pigments, thereby red fruit colour, to portray the importance of controlling pH of fruit during processing and storage for fruit products to appeal to consumers.

Anthocyanins are naturally occurring pigments of red and blue in fruits and flowers. In acidic conditions, anthocyanin provides a strong red pigment, while in basic conditions, they are a faint blue pigment (Wahyuningsih, et al., 2016). Higher intensity of red pigment of red fruits is more attractive for consumers as mentioned above. Therefore, it can be predicted that fruits with a higher concentration of red anthocyanin pigments will be more attractive for consumers. Drawing from this background, it can be hypothesised that for fruit in which its colour is determined by anthocyanin, the lower the pH, the more intense and appealing the red colour.

Anthocyanin Equilibria and pH

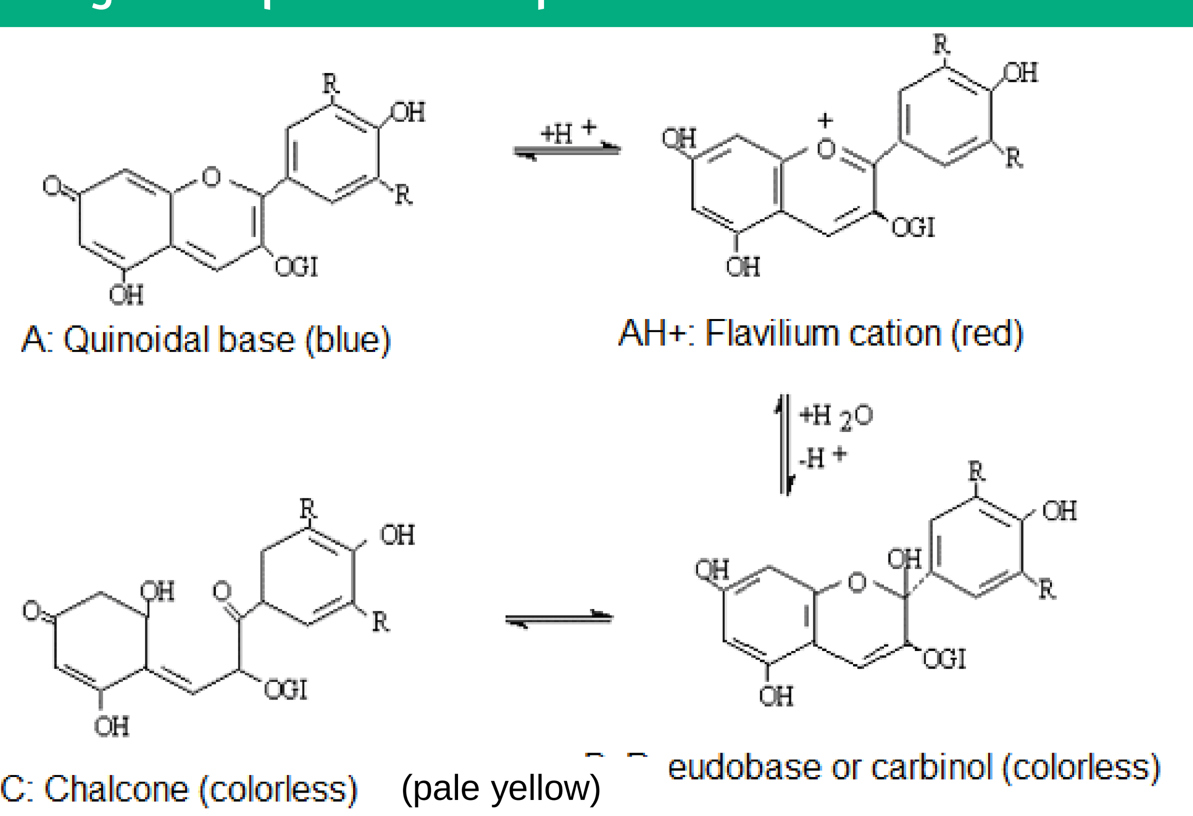

Figure 1: *Equilibrium reactions between four anthocyanin species* (Lopes et al., 2011)

As seen in figure 1, in an aqueous solution, anthocyanins are present in four different species. Multiple equilibria are present due to anthocyanin's high instability (Asenstorfer, 2001).

The H^+ concentration shifts the equilibrium positions in this system, altering the relative concentration of anthocyanin species in equilibrium which determines the colour of the solution. Le Chatelier's Principle states that if an equilibrium system is subjected to a change, the system will adjust itself to partially oppose the effect of the change.

When the equilibrium solution is red, anthocyanins are predominantly in the form of flavylium cations which are only stable in highly acidic solutions. i.e. At high H^+ concentration, anthocyanin is red. With increasing pH (decreasing H^+ concentration), according to Le Chatelier's Principle, the equilibrium will shift towards the quinoidal base direction. The concentration of the flavylium cation will decrease and the anthocyanin solution will become less red. The flavylium cation loses a proton and acts as a Bronsted-Lowry acid to form the blue quinoidal base which is an unstable pigment. The flavylium cation may also immediately hydrate to produce colourless pseudobases which isomerises (transform into a different chemical structure) to chalcone that has a pale yellow pigment. As a result, the solution will become less intensely red (Asenstorfer, 2001).

Research Data

In this depth study, the effect of pH on anthocyanin pigment (fruit colour) was investigated through secondary research. The pH that resulted in anthocyanin exhibiting the most intense red colour was also determined.

Jenshi roobha et al. (2011) investigated the effect of pH on anthocyanin extract pigment intensity in relation to time. The extracted anthocyanin pigments were exposed to fourteen different pH levels ranging from 1.1 to 10.5 and UV/Vis absorption spectra recorded at 520nm were accurately measured using a UV-visible spectrophotometer.

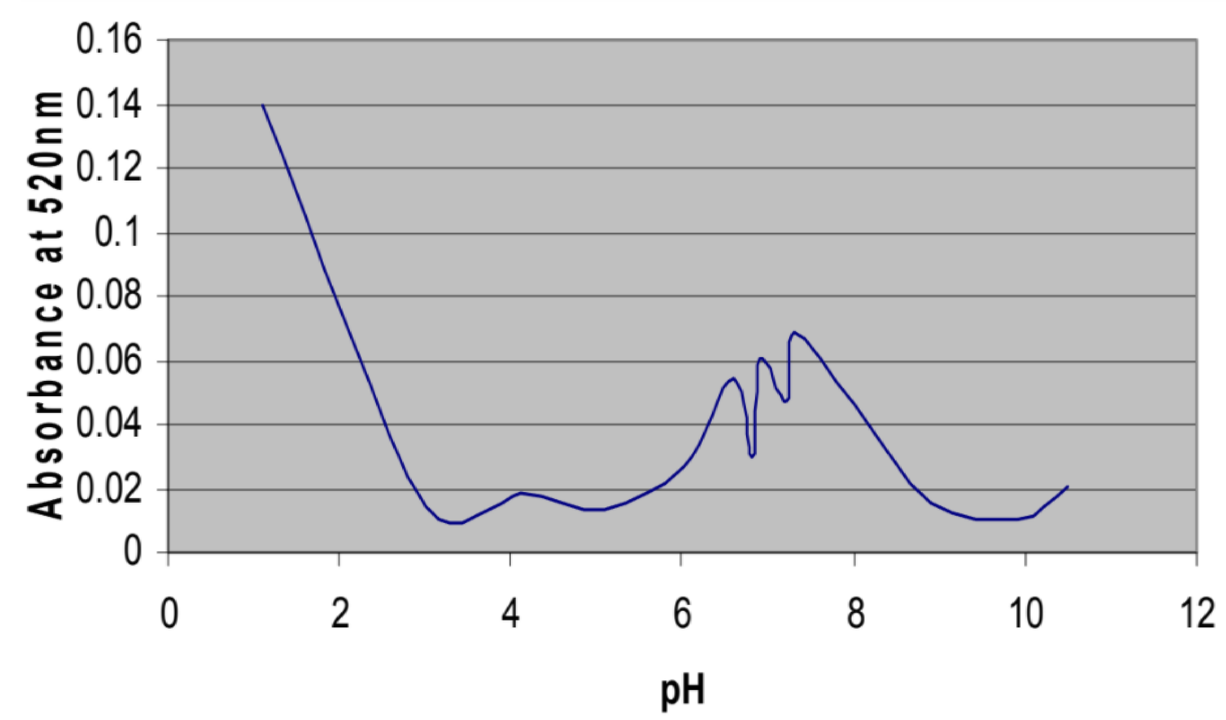

Figure 2: *Absorbance of Anthocyanin extract at various pH* (Jenshi roobha et al., 2011)

Absorbance is a measure of the quantity of light absorbed by a sample. Solutions with higher absorbance appear more intensely red coloured than solutions with lower absorbance. Jenshi roobha et al. (2011) presented that the absorbance of the anthocyanin sample peaks at a pH of around one as seen in figure 2. This conveys that the colour of anthocyanin is most intensely red at low pH.

They also observed that at a pH of 3.3, anthocyanin pigments had a pale pink colour and showed pale yellow colour at a pH of 10.5. Since solutions with lower colour intensity result in lower absorbance, figure 2 depicts that the absorbance at 520nm is lowest at these points. The slight fluctuations of absorbance between pH 6 and 8 indicate that the colour intensity oscillated and equilibrium position constantly shifted. This means that anthocyanins are the least stable at this range. Unstable anthocyanin pigments may lead to fruit discolouration (Andrés-Bello, et al., 2013). Thus, this investigation proved that the lower the pH the more intense the red colour of the anthocyanin pigment.

Reyes and Cisneros-Zevallos (2005) also concluded that acidic conditions were more optimal for red fruit colour. They investigated the effect of pH on the visual colour of aqueous anthocyanin extracted from red grapes. Red grapes have high anthocyanin content. The pH was accurately measured with a pH probe and red colour intensity was observed. The effect of pH was evaluated over 4 weeks by storing the extracts at different pH conditions (pH 1–10), at room temperature (25°C).

Figure 3: *Picture of aqueous anthocyanin extracts from grape at pH 1 to10 (left to right) after 28 days of storage* (Reyes and Cisneros-Zevallos, 2005)

The extracts from grapes showed red coloured solutions up to pH 3. For pH values from 4 to 7, extracts were a pale pink to yellow and further increase in pH from 8 to 10 yielded coloured extracts with pale green to yellow hues after 28 days as seen in figure 3. This is because in basic conditions where there is a decrease in the concentration of H^+, the blue pigments of anthocyanins become paler because the equilibrium between the four anthocyanin species shifts towards the colourless pseudobase and pale yellow chalcone direction to partially increase the concentration of H^+ (Andrés-Bello, et al., 2013). Thus, this proves that high pH levels are not optimal for red fruit colour with fruits that have anthocyanin pigments.

Furthermore, Holcroft and Kader (1999) studied the effects of controlled atmosphere storage on pH and their influence on anthocyanin colour expression in strawberries. Strawberries are fruits that are rich in anthocyanin pigment. Fruit storage in elevated CO_2 atmospheres is a commercial treatment for postharvest decay control. The strawberries were placed in jars that were connected to a continuous flow of CO_2, humidified at different atmospheres and were monitored. They measured the external colour quality of strawberries using a chromameter which allowed the accurate calculation of the intensity of colour saturation. The pH of external and internal tissues were measured using an Abbe refractometer. During their experiment, they discovered that the external colour of the controlled atmosphere stored strawberries darkened and became less intensely red in comparison to fruit stored in normal CO_2 atmospheres. This was because the pH of the internal tissues tended to increase with CO_2 treatment. Therefore, they concluded that controlled atmosphere storage adversely affected red fruit colour. Hence, this experiment displayed that increased pH of fruit leads to less intensity of redness in fruit.

Discussion

Secondary Investigations	pH	Colour
Jenshi roobha et al. (2011)	1	Highly intense red
	3.5	Pale pink
	10.5	Pale yellow
Reyes and Cisneros-Zevallos (2005)	1-3	Red
	4-7	Pale pink to yellow
	8-10	Pale yellow
Holcroft and Kader (1999)	Greater than 7	Low red intensity

Figure 4: *Tabular comparison between secondary research data's pH and resultant anthocyanin colour*

Secondary research data showed the effect of pH on anthocyanin pigment and therefore the colour of fruit. The data proved that the low pH of the fruits resulted in a higher red colour intensity. The results of the three studies showed similar and clear patterns of the relationship between pH and fruit colour as seen in figure 4.

The colour change of anthocyanin in the experiments portrayed that the colour of anthocyanin is determined by H^+ concentration in the anthocyanin solutions. This can be linked to the fundamental concept of Le Chatelier's Principle where colour change is dependent on the equilibrium position. As H^+ concentration (pH) increases, the equilibrium shifts towards the flavylium cation side to produce a red solution.

The reliability of the data was ensured by the all three studies portraying a consistent relationship between colour and pH; the lower the pH the more attractive the fruit colour. The information was highly accurate in that it followed the theoretical change in the colour of anthocyanin in relation to pH. To further improve accuracy, data that initially determined the anthocyanin percentage in the fruit used could be utilised and compared with fruit with a similar anthocyanin percentage since initial percentage of anthocyanin may be a control variable that may alter the results. The result of the depth study is only valid for fruits that have a high concentration of anthocyanin which affects their colour. It is not applicable for fruits where their pigment is not determined by anthocyanin. The secondary data obtained were from science journal articles written by professional scientists who conducted their experiments without bias. Appropriate methodology and measuring devices were evidently used for each investigation. Therefore, high validity of this secondary research is ensured.

The colour of anthocyanin is also affected by temperature and enzymatic activity (Andrés-Bello, et al., 2013). The relationship between pH and these variables may be a topic to be explored in future investigations.

Conclusion

In this depth study, the effect pH on anthocyanin pigment colour was successfully investigated and the optimal pH that produced the most intense red colour was determined. It proved that pH control of fruit is important in production and storage to create appealing bright red fruits for consumers. Results showed that the lower the pH, the more intense the red colour. Therefore the hypothesis was proved to be correct.

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