Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 11 (13): 2406-2410, 2011
ISSN 1812-5654 / DOI: 10.3923/jas.2011.2406.2410

The Effect of pH on Color Behavior of *Brassica oleracea* Anthocyanin

[1]Ummi Kalthum Ibrahim, [2]Ida Idayu Muhammad and [1]Ruzitah Mohd Salleh
[1]Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM),
40450 Shah Alam, Selangor, Malaysia
[2]Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering,
Universiti Teknologi Malaysia (UTM), 81310 Skudai, Johor, Malaysia

Abstract: Anthocyanins, as natural colorants are widely used in the food industry as an alternative to synthetic colorants due to their health benefits, beautiful color and safe to be consumed. Despite of the current challenge to maintain their color properties, the instability of the color can be utilized as an indicator in the food packaging industries. One of the factors which affect the color of the anthocyanins is the level of pH. In the food industry the anthocyanins color can easily change due to the various pH condition of the food product. Increasing the pH and temperature, during processing and storage would increase the degradation rates of anthocyanins. This study focuses on the anthocyanin color behavior at various pH in the liquid and solid phase. Samples of anthocyanin in aqueous solutions were studied at various pH levels between 1.0 to 14.0 at a period of 10 days. Colors were expressed by the CIELAB coordinates, color tone, color intensity and color lightness. Powdered anthocyanin exhibits more stable compared to juice anthocyanin at most pH values, showing no changes in color intensity and color tone and little changes in color lightness. The variation in the results suggested that further developments of anthocyanin as a potential pH color indicator in food packaging system are required.

Key words: Anthocyanin, *Brassica oleracea*, color stability

INTRODUCTION

There has been an increased interest in the development of food colorants from natural sources as alternatives to synthetic dyes. Anthocyanins are the naturally occurring, water-soluble compounds that impart many of the orange, red, magenta, violet, purple and blue colors to a variety of fruits, vegetables and plants. Plants rich in anthocyanins are blackberry, blackcurrant, chokeberry, cherry, eggplant, blue grape, vaccinium and red cabbage. The interest in anthocyanins derives not only from their coloring effect but also from their beneficial properties, including antioxidising activity, improvement in the tightness of capillary blood vessels and prevention of thrombocyte aggregation, all of which reduce the risk of circulatory diseases (Walkowiak-Tomczak and Czapski, 2007). Despite the great potential of application that anthocyanins represent for food, pharmaceutical and cosmetic industries, their use has been limited because of their relative instability. Color stability of anthocyanin influenced by many factors include structure and concentration, pH, temperature, light, presence of co-pigments, self association, metallic ions, enzymes, oxygen, ascorbic acid, sugar and their degradation products, proteins and sulphur dioxide (Ersus and Yurdagel, 2007).

The role of anthocyanins as food coloring agents becomes very important since they form the reds and the blues of many fruits and vegetables and provide the attractive color of many fruit juices, wines, jams and preserves (Fossen *et al*., 1998). Their color is easily affected by a number of reactions occurring in food products and the major problem associated with the storage of anthocyanins is their instability caused by pH, storage temperature, chemical structure, concentration, light, oxygen, solvents the presence of enzymes, flavonoids, proteins and metallic ions. The main focus of this study is to determine the color behavior of anthocyanin towards various pH levels. Generally, increasing of pH during storage increased the degradation rates of anthocyanins.

Anthocyanins can be found in different chemical forms which depend on the pH of the solution as shown in Fig. 1 (Castaneda-Ovando *et al*., 2009). At pH 3 or lower, the flavylium cation is the predominant species and contributes to purple, orange and red colors. As the pH increase, kinetic and thermodynamic competition occurs between the hydration reaction of the flavylium cation

Corresponding Author: Ummi Kalthum Ibrahim, Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia Tel: +603-55436357 Fax: +603-55436300

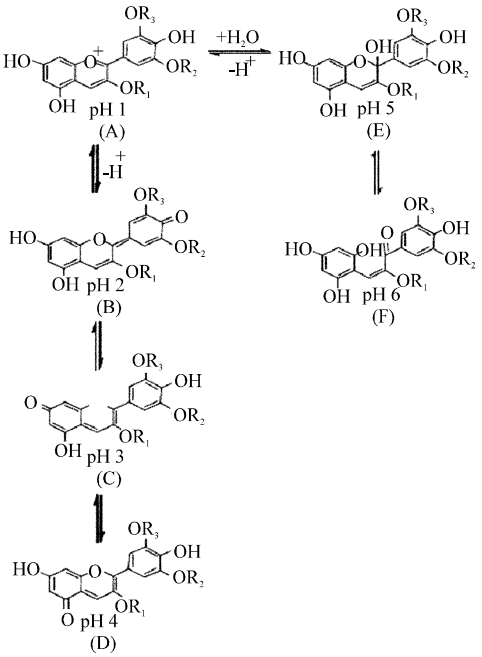

Fig. 1: Chemical Forms of Anthocyanin at Various pH (Castaneda-Ovando *et al*., 2009)

and the proton transfer reactions related to its acidic hydroxyl groups. While the first reaction gives colorless carbinol pseudo-bases (pH 5) which can undergo ring opening to yellow retro-chalcones (pH 6), the latter reactions give rise to more violet quinonoidal bases (pH 4). Further deprotonation of the quinonoidal bases can take place at pH between 6 and 7 with the formation of more bluish resonance-stabilised quinonoid anions. At pH values higher than 7, the anthocyanins are degraded depending on their substituent groups. It is well known that anthocyanin properties, including color expression, are highly influenced by anthocyanin structure and pH. According to Torskangerpoll and Andersen (2005), the parameters employed for describing color variation of anthocyanin solutions have mainly been shifts of the visible λ_{max} as a measure for hue variations and absorptivity changes for variations of color intensity.

Color is an important factor influencing consumers' acceptability of food products. This is due to the fact that consumers always associate food color with other qualities such as freshness, ripeness and food safety. However, natural food colorants are not stable in food products. They can be decolorized and degraded due to many factors as mentioned above. There are many techniques in order to improve the color stability of anthocyanins. Co-pigmentation, compartmenting, encapsulation and association are considered to cause stabilization of the anthocyanins (Heins *et al*., 2001).

Color is an important feature of agricultural and food products due to its close association with the perceived quality of the product. It is a widely used parameter in the assessment of their maturity, freshness, nutritional condition and growth factors. At the present time, the color judgment of agricultural and food products still depends mainly on human eyes and brain. These are superbly sensitive organs but the judgment is subjective and inevitably affected by human physical condition and environmental elements. Modern instruments are necessary to provide consistent measurement and quantitative expression of color (Zhang *et al*., 1998). Methods available for color measurement can be categorized into three broad types; visual systems, tristimulus colorimetry and spectrophotometry. Visual systems involve comparison with colored references under controlled illumination, tristimulus instruments use three filters to provide the red, green and blue components of a color and the spectrophotometric method measures color by determining a reflection or transmission spectrum (Zhang *et al*., 1998).

CIE L*a*b* (CIELAB) is the most complete color model used conventionally to describe all the colors visible to the human eye. It was developed for this specific purpose by the International Commission on Illumination (Commission Internationale d'Eclairage). The asterisk (*) after L, a and b are part of the full name, since they represent L*, a* and b*, derived from L, a and b. CIELAB is an Adams Chromatic Value Space. The three parameters in the model represent the lightness of the color (L* = 0 yields black and L* = 100 indicates white), its position between magenta and green (a*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow).

MATERIALS AND METHODS

Experimental procedures: The materials used were powdered anthocyanins from red cabbage (*Brassica oleracea*) and juice obtained by extracting crude red cabbage employing solvent extraction technique. In this study, it can be divided into three parts which were extraction of anthocyanin from crude red cabbage, preparation of buffer solutions for various pH and color measurement for both powdered and juice anthocyanin for acidic, neutral and basic conditions.

Anthocyanin extraction: Red cabbage leaves were shredded and soaked in 20% ethanol/water for 24 h. Then it was boiled for 5-10 min until purple color appeared.

Buffer solution preparation: Buffer solutions for 14 different pH values were prepared. The accurate pH for each buffer solution was measured with a portable pH Meter. The spectral behavior of anthocyanins is dependent on pH and substances present in the solutions may influence the color.

Color analysis: The effect of pH on both anthocyanin juice and powdered was studied at pH 1 (acidic), pH 7 (neutral) and pH 13 (basic). Samples were kept in screw-capped bottles and shaken continuously in controlled temperature. The absorbance of the solution at 380 nm was monitored and degradation of cabbage red color was determined. A HACH DR/4000 U spectrophotometer (model HACH PROGRAM 1666) was used to measure the color using CIELAB color scale method. CIELAB parameters (L*, a* and b*) at same wavelength, λ (380nm) was recorded from the spectrophotometer. The color behavior was determined using CIELAB system L*, C*and H^0. L* designates the color lightness, C* is the color intensity and H^0 is the color tone. Color parameters were recorded every two days at a period for 10 days. Samples were kept in a refrigerator (4°C) between the measurements.

RESULTS AND DISCUSSION

Color of anthocyanin: By boiling the red cabbage leaves, a class of pigment molecules called anthocyanins will dissolve into the liquid. Anthocyanin molecules will change their color depending upon the pH of their environment thus it may serve as a pH indicator. The anthocyanin turns red-pink in acids (pH 1-6), reddish-purple in neutral solutions (pH 7) and green in alkaline or basic solutions (pH 8-14) (Fossen *et al*., 1998). A change of red cabbage color solutions was observed when it was exposed to various pH conditions. The effect of pH on the sample can be determined by the CIELAB parameter values through the color of the solution.

Color characteristics of the samples: Quantitative color measurements have been carried out by spectrophotometry in solutions of anthocyanins previously isolated from red cabbage. From the spectrophotometry readings, chromatic analyses were employed to calculate the intensity and color lightness of the sample following the CIE (Commission International de l'Eclairage) system of 1976 (Goncalves *et al*., 2007). Values of L*, a* and b* were measured to describe a three dimensional color space and interpreted as follows: L* indicates lightness read from 0 (completely opaque or ''black'') to 100 (completely transparent or ''white''). A positive a* value indicates redness (-a* is greenness) and a positive b* value yellowness (-b* is blueness).

Hue (H^0) is color tone whether it is blue, red, green, yellow and etc. Chroma (C*) is color intensity whether it bright or dull and Lightness (L*) is the amount of color whether it light or dark. Equation 1 and 2 represent the equation for H^0 and C*, respectively which were calculated by the transformation of a*and b* values:

$$\text{Color tone } H^0 = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$\text{Color Intensity } C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (2)$$

Based on the hue circle, H^0 values are stepped from 0 to 360° (red). The other reference values are 90° (yellow), 180° (bluish green) and 270° (blue) (Ersus and Yurdagel, 2007). Differences in hue angle (H^0) could be attributed to both differences in anthocyanin and phenols composition (Reyes and Cisneros-Zevallos, 2007). Changes in the H^0 parameter describe the direction of changes of the color tone. Generally, during storage, H^0 value increased with an increase in the level of independent variables (storage time). This indicates the change of the color towards yellow (high pH) which is associated with the development of products of anthocyanin degradation. Color intensity (color purity) indicates if color is pure or contaminated by the presence of another pigment (Gonnet, 1999). The loss of lightness was reflected by a reduction of L* and was directly related to the humidity during storage (Heredia *et al*., 1998).

According to the CIELAB parameters storage, their color stabilities depend highly on pH and anthocyanin structure. Juice anthocyanin was found to be significantly more brighter color than powdered anthocyanin at most pH values, showing very high L* and C* values after storage for 10 days (Fig. 2 and 3). The maximum color intensity (C*) for both juice and powdered anthocyanin was observed in the alkaline region at pH 13. From the result, it indicates that powdered anthocyanin color intensity was consistently stable compared to juice anthocyanin at a wide pH range but the color lightness (L*) was slightly decreased with increasing storage time. This is due to degradation of anthocyanins.

From the analyses, H° values of both powdered and juice anthocyanin samples have reddish color at the pH 1. Increasing the pH values from 1 to 7, the color gradually changed toward more purplish tones. At higher pH values, the hue angles decreased dramatically indicated by the changes of color to green. As revealed in Fig. 4, there were no changes in hue values for powdered anthocyanin and small changes for juice anthocyanin at

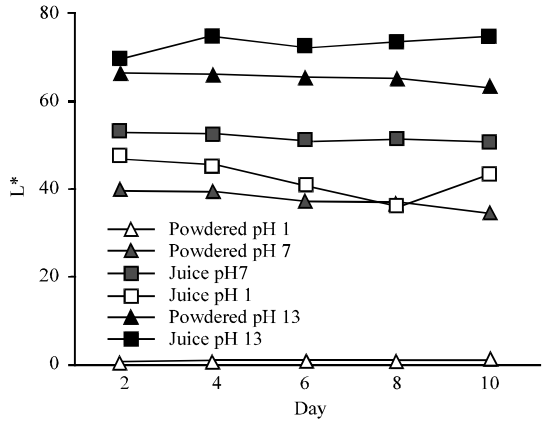

Fig. 2: Color lightness of powdered and juice anthocyanin at various pH

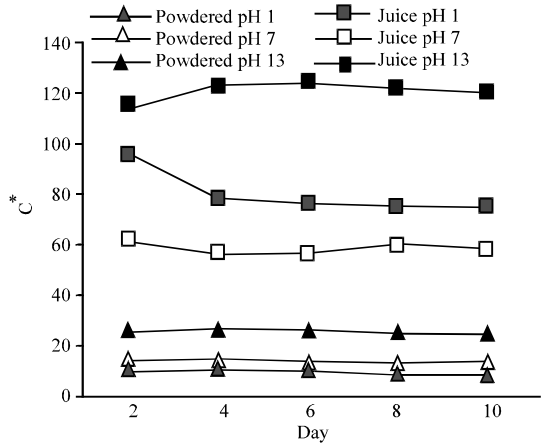

Fig. 3: Color intensity of powdered and juice anthocyanin at various pH

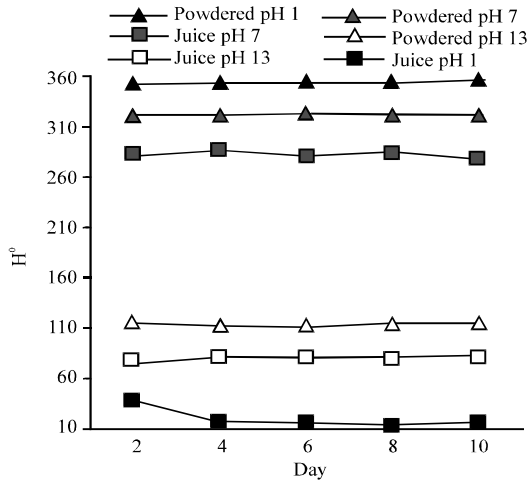

Fig. 4: Color tone of powdered and juice anthocyanin at various pH

various pH after storage for 10 days. This suggests that powdered anthocyanin have relative stable hue compared with juice anthocyanin.

Generally, powdered anthocyanin showed greater color stability, expressed by H°, C* and L* parameters than juice anthocyanin at all pH values.

CONCLUSION

In conclusion, anthocyanin occurred in the most intense reddish color in acidic aqueous solutions. Neutral solutions will result in a purplish color and basic solutions will appear in the green-yellow region of color. Powdered anthocyanin is more stable than juice anthocyanin at most pH values, showing no changes in C* and H^0 and little change in L* values at a period of 10 days storage.

Powdered anthocyanins have better color stability at specific pH which allows it to be used as pH color indicator in food packaging. Because of its wide range of color tones in relation to pH, as well as high color stability, anthocyanin extracted from red cabbage is also highly recommended for food colorant. The color variations exhibit from the results obtained emphasize the importance of future work to develop anthocyanin as pH color indicator in food packaging system.

ACKNOWLEDGMENTS

Thanks are due to all who are involved in this work for their guidance and support.

REFERENCES

Castaneda-Ovando, A., M. de Lourdes Pacheco-Hernandez, M.E. Paez-Hernandez, J.A. Rodriguez and C.A. Galan-Vidal, 2009. Chemical studies of anthocyanins: A review. Food Chem., 113: 859-871.

Ersus, S. and U. Yurdagel, 2007. Microencapsulation of anthocyanin pigments of black carrot (*Daucuscarota* L.) by spray dryer. J. Food Eng., 80: 805-812.

Fossen, T., L. Cabrita and O.M. Andersen, 1998. Colour and stability of pure anthocyanins influenced by pH including the alkaline region. Food Chem., 63: 435-440.

Goncalves, B., A.P. Silva, J. Moutinho-Pereira, E. Bacelar, E. Rosa and A.S. Meyer, 2007. Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). Food Chem., 103: 976-984.

Gonnet, J.F., 1999. Colour effects of Co-pigmentation of anthocyanins revisited-2. A colorimetric look at the solutions of cyanin Co-pigmented by rutin using CIELAB scale. Food Chem., 66: 387-394.

Heins, A., H. Stockmann and K. Schwarz, 2001. Designing Anthocyanin-Tailored Food Composition. In: Biologically Active Phytochemicals in Food: Analysis, Metabolism, Bioavailability and Function, Pfannhauser, W., G.R. Fenwick and S. Khokhar (Eds.). Royal Society of Chemistry, UK., pp: 378-381.

Heredia, F.J., E.M. Francia-Aricha, J.C. Rivas-Gonzalo, I.M. Vicario and C. Santos-Buelga, 1998. Chromatic characterization of anthocyanins from red grapes-pH effect. Food Chem., 63: 491-498.

Reyes, L.F. and L. Cisneros-Zevallos, 2007. Degradation kinetics and colour of anthocyanins in aqueous extracts of purple and red flesh potatoes (*Solanum turberosum* L.). Food Chem., 100: 885-894.

Torskangerpoll, K. and O.M. Andersen, 2005. Colour stability of anthocyanins in aqueous solutions at various pH values. Food Chem., 89: 427-440.

Walkowiak-Tomczak, D. and J. Czapski, 2006. Colour changes of a preparation from red cabbage during storage in a model system. Food Chem., 104: 709-714.

Zhang, J., S. Sokhansaj, S. Wu, R. Fang, W. Yang and P. Winter, 1998. A transformation technique from rgb signals to the munsell system for colour analysis of tobacco leaves. Comput. Electron. Agric., 19: 155-166.