A COMPARATIVE STUDY ON THE DEGRADATION KINETICS, PIGMENT STABILITY AND COLOUR CHARACTERISTICS OF JUICE MODEL SYSTEMS COLOURED WITH PURPLE YAM AND RED CABBAGE ANTHOCYANINS

Anumol Jose[1], Anilkumar Madhavan Nair*[1]
[1]Department of Botany, Union Christian College, Aluva , Kerala, India, Phone: 0484 2609194
*E-mail: drmakumar@gmail.com

ABSTRACT
Red cabbage and purple yam are considered as rich sources of acylated anthocyanin compounds which are important in the natural food colourant industry due to their unusual storage stability. In this study pigment stability and color characteristics of model juice systems colored with anthocyanin extract from purple yam and red cabbage were evaluated for a period of six months(27 weeks) under two different storage temperatures. Reflectance spectrometric analysis and colour quantification in terms of CIELab coordinates were adopted for the present study. Color characteristics of juice model systems coloured with anthocyanins were comparable to the colour tone produced by synthetic colourant FD&C red # 3.Stability traits like pigment retention percentage, monomeric anthocyanin content and polymeric color percentage were measured spectrophotometrically and half-lives of pigments at different storage temperatures were calculated. Storage temperature influenced the stability and color characteristics of purple yam and red cabbage anthocyanins. At room temperature, purple yam anthocyanins showed higher pigment retention and stable color characteristics than red cabbage anthocyanins. Refrigeration improved half-life of purple yam anthocyanin for up to 141 weeks. Results suggested the applicability of purple yam anthocyanins as a stable alternative to the red artificial colorant in juice systems.

Keywords: Anthocyanin, Purple yam, Red cabbage, juice model system, pigment stability, storage temperature, natural colorant

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1. INTRODUCTION

Color is an important aspect which contributes to the acceptability of commercial products such as foods, cosmetics, textiles, and pharmaceuticals. The red color is an inevitable component in the food industry for commodities like juices, soft drinks, sweets, candies, and jellies. Synthetic colorants such as Erythrosine, Allura red, and Ponceau4R are the red/pink food colors legally permitted all over the world (Scotter, 2003). The amount of synthetic colorants in food items often exceeds the maximum allowable concentration (Dixit *et al*., 2013; Tsai *et al*., 2015). Health problems emerging due to the continuous use of these synthetic food colorants is a matter of concern among people for ages. Several studies pointed out the correlation between use of synthetic colorant and increase in hyperactive behavior among children (Bateman *et al*., 2004; Feingold, 1975; Kobylewski, 2012). An alternative to this problem is the use of natural colorants in food items. Natural colors include pigments derived from plant, animal and mineral sources. One of the plant pigments that have immense potential to act as a red/pink natural colorant is anthocyanins. Anthocyanins are a class of polyphenolic compounds which are responsible for the blue to red colors in the plant kingdom. More than 400 types of anthocyanins have been discovered and described so far (Giusti *et al*.,1999). Chemical structure of anthocyanin undergoes a pH-dependent reversible transformation in aqueous solutions. In acidic pH, anthocyanins are red flavylium cations and they shift to blue colored quinoidal base forms at near neutral pH. The most important factors that contribute to the color characteristics of anthocyanins are the

type of compounds and the extent of acylation in its structure (He J, 2010).

A variety of fruits and vegetables like red cabbage, red sweet potato, red potato, black carrot, red radish, blackberry, Acai berry and several others are reported to possess commercially important anthocyanin compounds and their chemical structures are also characterized (Ongkowijoyo *et al*.,2018). Yet researchers and food chemists are in search of an easily available and economically viable source of anthocyanins. Anthocyanins from different sources vary in their stability and color characteristics which in turn influences their applicability in food systems. Potential of anthocyanin as an alternative to red synthetic food colorant was proven in several food model systems like yogurt, juice and soft drink (Wallace, 2008; Brenes*et al*.,2005; Dyrby*et al*.,2001). But some factors which limit applications of anthocyanins in food systems are insolubility, insufficient hue, incompatibility with food matrix and instability of colour (Henry,1996). So when new anthocyanin sources are reported their applicability studies in food systems are inevitable to understand their full potential.

Purple yam (*Dioscorea alata* L.) is an underutilized tropical crop known for its edible purple fleshed tuber and has been proven as a rich source of acylatedanthocyanins (He H *et al*., 2015; Shoyama *et al*., 1990; Zhang *et al*., 2018). This particular class of anthocyanins is reported to be more stable and has already gained importance in the natural food color industry (Bakowska-Barczak, 2005). Yet no studies have been reported so far regarding the applicability of purple yam anthocyanins in food systems. Red cabbage (*Brassica oleracea* L.) is also a well-known vegetable source of anthocyanin with high yield per unit area (Piccaglia*et al*., 2002). It has been identified as a reservoir of highly acylated anthocyanins (Charron *et al*., 2007). Out of two acylated anthocyanin sources, purple yam is least studied regarding the pigment stability and color characteristics in food model systems.

Present study focus on comparing the storage stability and color characteristics of purple yam and red cabbage anthocyanins in juice model systems with an emphasis on its degradation kinetics. This study also aims to investigate the influence of storage temperature in the degradation of monomeric anthocyanins and CIELab color coordinates of juice systems over time.

2. MATERIAL AND METHODS

Materials: purple yam tubers (*Dioscorea alata*, land race -Chora kachil) and red cabbage heads (*Brassica oleraceae* variety -Questo) used in this study were collected from local farmers of Kerala, India. All the chemicals used in this study were of analytical grade from Merck (Germany).

Extraction of anthocyanin: Extraction of anthocyanins was performed according to the protocol of Wu and Ronald (2006) with some modifications. The solvent used for extraction was 60% methanol acidified with citric acid (0.2% m/V). Red cabbage head and purple yam tubers were sliced into small pieces and homogenized using a tissue grinder. Extraction solvent (10ml/gm of tissue) were added to the tissue homogenate and incubated for one hour at 4^0C.The supernatant was collected by filtration and the process was repeated thrice. All the filtrates were pooled and concentrated with a rotary vacuum evaporator at 37^0C to obtain an enriched fraction of anthocyanin.

Purification of anthocyanin: The enriched fraction of anthocyanin extract was further purified in Sep Pak C18 cartridge (Bond elute, Agilent Technologies). The cartridge was activated with methanol followed by sample loading and washing with ethyl acetate and acidified water (0.1% m/V phosphoric acid) sequentially. Anthocyanin fraction recovered from the column using acidified methanol and concentrated by rotary evaporation

Preparation of Juice model system: Juice model system was prepared according to the formulations of Rodríguez-Saona *et al.* (1999) with some modifications. A 15°Brix solution containing 15% (m/V) sucrose, 0.1% (m/V) potassium sorbate, 0.1%(m/V) sodium benzoate and 0.2% (m/V) citric acid. The

solution was transferred to 20ml glass bottles to which 15mg each of red cabbage and purple yam anthocyanins were added separately to color the solutions. All the preparations were in triplicates and the number of vials was according to the number of sampling required during storage. The pH of the solutions was adjusted to 3.5 using sodium bicarbonate, and glass vials capped and pasteurized in the water bath at 85^0C for 25 min. The vials were stored in different temperatures (room temperature and refrigeration) for 27 weeks. The pH, total dissolved solids, and Brix of juice solutions were measured periodically and any change was rectified accordingly. A reference model juice solution was prepared using FD&C red #3 as the colorant. The concentration of artificial food colorant in juice solutions was 150ppm.

Quantification of Monomeric and polymeric anthocyanin: Total monomeric anthocyanin content was determined by using pH differential method (Giusti *et.al* 2005). Aqueous anthocyanin extract was incubated in pH 1.0 and pH 4.5 (0.45 M sodium acetate) buffers for 15 min. Absorbance was taken at 520 nm and 700 nm in a UV−visible Spectrophotometer 2400 (Shimadzu, Japan). Monomeric anthocyanin content (mg/L) was calculated using the equation formulated by Giusti and Wrolstad (2001). Concentrations of monomeric anthocyanin were expressed as cyanidin 3-glucoside equivalents.

Polymeric anthocyanin was measured by bisulfite method (Giusti and Wrolstad, 2001). Samples and control solutions were bleached with 2% potassium bisulfite solution and absorbance were taken at 420 nm and 520nm. Polymeric anthocyanin content was calculated using the equations proposed by Giusti and Wrolstad (2005).

Pigment retention %

The absorbance of juice solutions at 520nm was taken at different time points of storage and percentage retention of anthocyanins was calculated usingEquation

Pigment Retention(%)
= Absorbance after storage
÷ Absorbance before storage × 100

Calculation of degradation kinetic parameters: Degradation of anthocyanin is modelled using first-order equation Ct = C0 exp (-kt) where Ct and C0 are the anthocyanin concentrations (mg /L) at time t and t0, respectively, k is the first order kinetics constant and t is the storage time in weeks. A Linear regression plot analysis was applied to calculate the degradation rate constants (k). Natural logarithm of the anthocyanin concentration was plotted against various storage times, and the rate constants (k) were determined from the slopes. Half-life value (t1 /2) of anthocyanin content was calculated by using the equation $t1/2 = (ln\ 1/2)/k$.

Color characteristics: The color coordinates of the samples were measured in a UV visible spectrophotometer with ISR-2600 Plus Integrating Sphere attachment and color analysis software (Shimadzu). CIELAB color space coordinates of L*(luminosity), a* (intensity of red and green) and b* (intensity of yellow and blue) chroma (C*) and hue angle (H) were calculated from the reflectance spectra.

Statistical analysis: All the experimental results were expressed as the mean ± standard deviation of three replicates and were subjected to one-way analysis of variance (ANOVA). Post-hoc analysis by Tukey HSD was performed when required. P values ≤ 0.05 were regarded as significant. All the statistical analysis were performed in SPSS software (version 20).

3. RESULTS AND DISCUSSION

Absorption spectrum

Absorption spectra of juice solutions colored with red cabbage and purple yam anthocyanin were comparable to red artificial colorant FD & C red # 3. Absorbance maxima of three juice model solutions were in the range of 505-530nm (Fig 1). Juice solution colored with artificial color produced an absorption peak at 508nm. Absorption maxima of purple yam were on 524nm while red cabbage showed an absorption peak at 528nm.

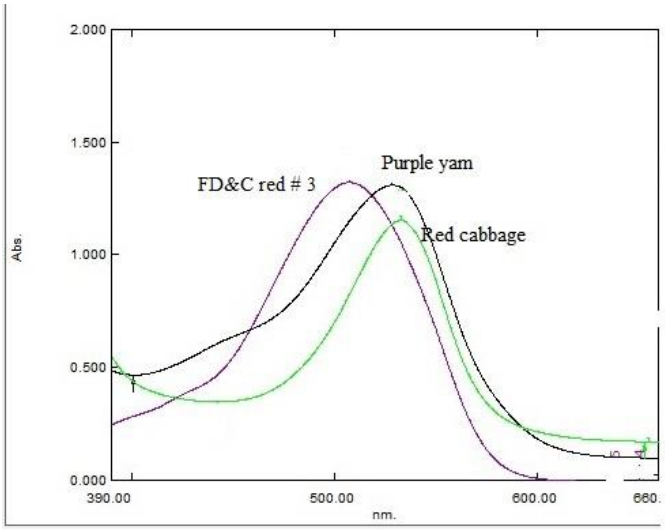


Fig. 1: Comparative Absorption spectra of juice systems colored with red cabbage, purple yam and synthetic color FD&C red # 3

Table 1: CIELab color coordinates of juice model systems

Nature of colorant	Concentration PPM	L*	a*	b*	C*	H (Hue angle)
FD&C red# 3	150	66.63±2.6	14.01±1.0	1.31±0.6	14.07 ±1.2	5.35±0.2
Purple yam anthocyanin	400	67.58±1.2	48.09±1.1	18.13±0.8	51.39±2.1	20.65±0.56
Redcabbage anthocyanin	400	60.29±2.9	53.67±0.7	-1.64±0.5	53.7±0.4	356.5±0.4

Note: values are mean ±S..D(n=3)

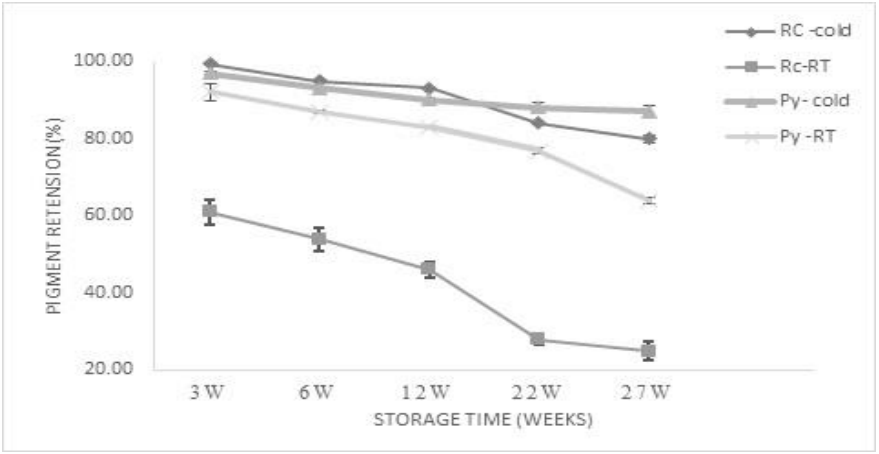


Fig. 2: Comparison on the pigment retention of purple yam and red cabbage anthocyanins over different storage conditions

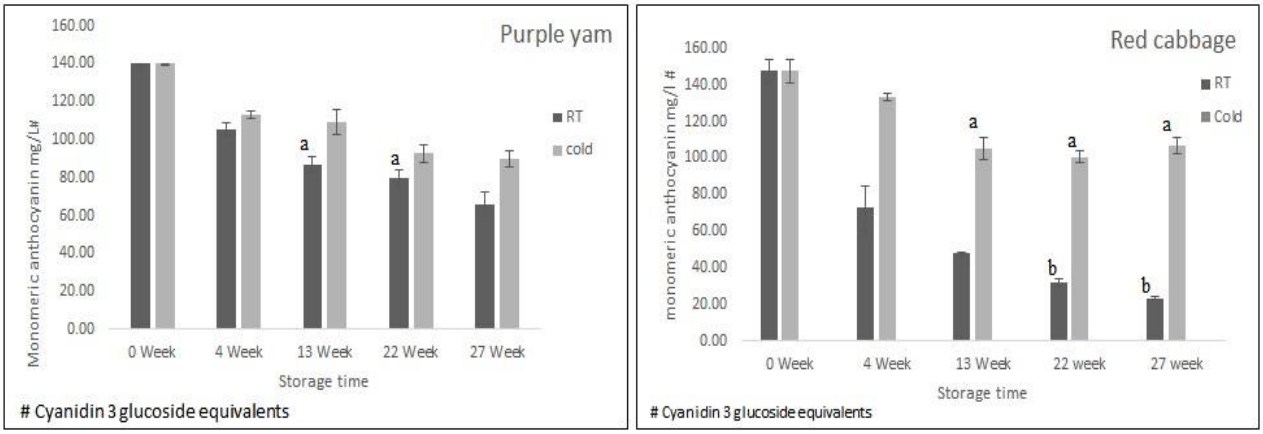
Note : The vertical bars in the graph are standard errors of the mean (± SD). (RC - Red cabbage, Py - Purple yam, RT- Room Temperature)

Pigment stability in juice model system

Pigment retention percentage : Pigment retention percentage of anthocyanins in juice systems shows a decreasing trend over a time period of 27 weeks (Fig 2). Juice colored with purple yam anthocyanins showed 87% pigment retention after six months of storage under refrigerated condition. In the room temperature this retention percentage was reduced to 67%. In the case of red cabbage, pigment reduction was relatively higher in both storage conditions. Juice stored under refrigeration retained 80% of red cabbage derived anthocyanins while at room temperature the degradation of pigment was high and retention percentage was only 25% after 6 months.

Changes in Monomeric anthocyanin content

Monomeric anthocyanin content in both juice systems showed a gradual reduction over time (Fig 3). Results of the present study shows that refrigerated temperatures improve monomeric anthocyanin stability in juices. At room temperature, monomeric anthocyanin content in red cabbage is gradually decreasing and reach up to 25 mg over six months of storage time while refrigeration reduced the degradation. Degradation of monomeric anthocyanin content in purple yam anthocyanin colored juices is relatively less and more than 80% of the initial concentration is preserved under refrigeration. After 27 weeks of storage in room temperature fifty percentage of monomeric anthocyanins are preserved in the purple yam based juice model.



Data are means (n=3)+SE.Difference between bars connected by same letters are not significant ($p< 0.05$)

Fig 3: Changes in monomeric anthocyanin content in juice model systems over time

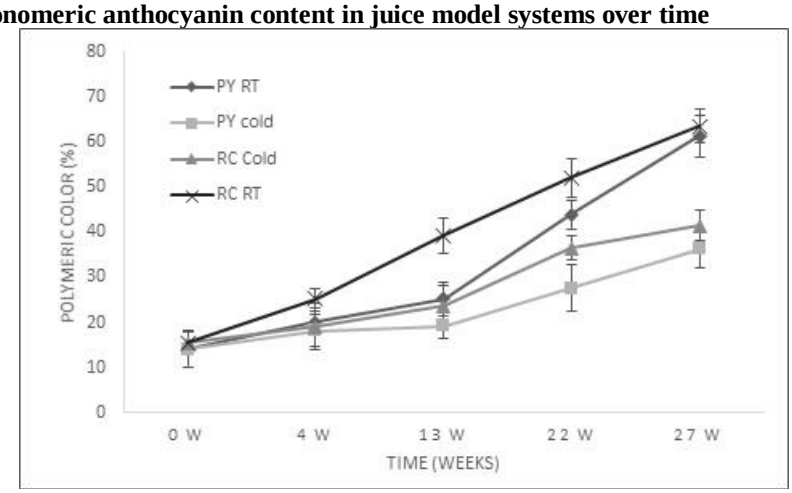


Fig. 4: Comparative Polymeric color % in juice model system

Note: Data are represented as mean ±SD (no:3)

Changes in Polymeric %

Percentage of polymeric color in juice model system shows variations according to the storage temperature and type of anthocyanins in them. Anthocyanin degradation and the constitutive formation of colored polymeric compounds were higher in juices stored in room temperature. After 27 weeks of storage in room temperature it is observed that sixty percent of the color intensity was contributed by polymerization of anthocyanin pigment. Both purple yam and red cabbage showed similar degradation trend and polymeric color percentage in room temperature. The extent of polymeric color formation in juices stored in cold temperature was twenty percent lesser.

Degradation Kinetics

Storage temperature had a profound influence on degradation kinetics of anthocyanin content in model juices (Table 1). The degradation rate of anthocyanin under room temperature storage was higher when compared to refrigerated temperatures. In both storage conditions, higher stability of total anthocyanin and monomeric anthocyanin were obtained for juice colored with purple yam extract. The half-lives of (t1/2) of purple yam derived total anthocyanin and monomeric anthocyanin in juice stored under room temperature was estimated as 41 and 24 weeks respectively while in the case of red cabbage it was 14 and 11 weeks. Refrigeration increased the extent of anthocyanin stability with estimated of over a year for purple yam (141 weeks) and 90 weeks for red cabbage anthocyanin colored juice.

Changes in CIELabcolor coordinates

Data are represented as mean±SD (RC -Red cabbage, Py-Purple yam, RT- Room Temperature)

Color stability in terms of lightness (L*) were gradually increased in all samples and storage temperature do has an influence on this. (Fig 5a). Juice colored with red cabbage anthocyanin stored in room temperature showed a sharp increase in lightness while in all the others lightness increased only about 10 degrees. Chroma or color strength was also influenced by storage temperature and type of anthocyanin present in juices. Purple yam anthocyanin colored juice kept under refrigeration maintained the color intensity up to six months. Reduction in chroma was more in juice colored with red cabbage anthocyanin.

Table 2: Degradation Kinetics parameters of purple yam and red cabbage anthocyanins at different storage temperatures in juice model systems

Source	Storage Temperature	Type of compound	K (Days -1)	t1/2 (weeks)#	R2
Red cabbage	Room temp	Total anthocyanin	6.8x 10-3	14	0.955
		Monomeric anthocyanin	8 x 10-3	11	0.94
	Cold storage	Total anthocyanin	1.1 x 10-3	90	0.96
		Monomeric anthocyanin	2.2 x 10-3	45	0.91
Purple yam	Room temp	Total anthocyanin	2.4x 10-3	41	0.94
		Monomeric anthocyanin	4.2x 10-3	24	0.094
	Cold storage	Total anthocyanin	7x 10-4	141	0.928
		Monomeric anthocyanin	2.1 x 10-3	47	0.904

Note : # Half-lives are predicted assuming the degradation of anthocyanins follows the same pattern as obtained with the regression model after 27 weeks of storage.

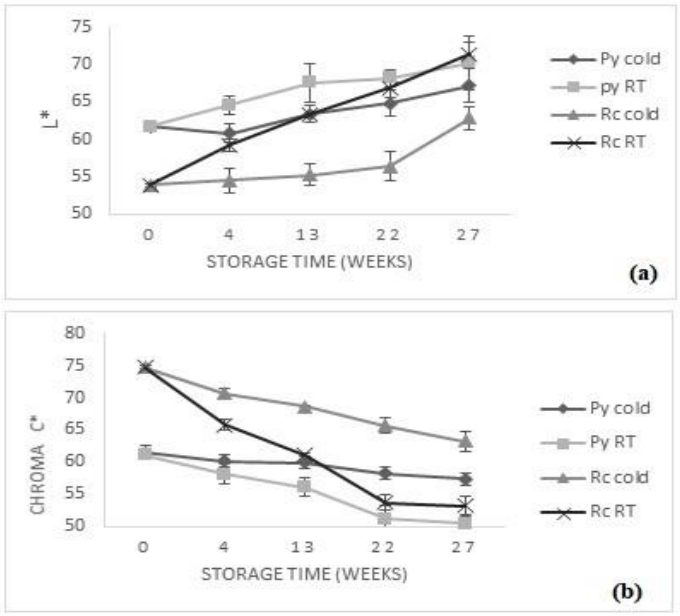


Fig. 5: Changes in L* (a) and chroma (b) of model juice systems colored with purple yam and red cabbage anthocyanin during storage

Discussion

Absorption spectra and CIELab color coordinates showed that purple yam and red cabbage anthocyanins produced comparable color tones to FD&C #3. However s the concentration of anthocyanins needed to produce a comparable colourtone to artifical colourant was much higher. Red cabbage anthocyanins produced red color with a blue hue (negative b* value and hue angle close to 360) while purple yam produced red color with yellow hue (positive b* value and hue angle 20). The red tone produced by FD&C #3 in juice system was the true red color with hue angle close to zero and positive b* value (Itle , 2009). The blue tone in red cabbage extract could be explained by the higher proportions of diacylated pigments present in it (Ahmadiani et al., 2014). Cyanidin-3,5-diglucoside and cyanidin-3-sophoroside-5-glucoside are reported to be the major anthocyanins present in red cabbage (Dyrby et al., 2001). Cyanidin-3,5-diglucoside considered as the blue pigment because of the diacylation at C3 and C5 regions which shifts its color to blue hues in all pH (Sigurdson, 2018). In purple yam tuber extract seven acylated anthocyanins were reported so far and alatanin C (Cyanidin 3-(6-sinapoylgentiobioside) counts for about 46.3% of the total anthocyanins (He H et al., 2015; Moriya et al., 2015). Alatanin C is a monoacylated anthocyanin with an absorption maxima of 530nm. The reddish tone in purple yam anthocyanins could be explained by the abundance of alatanin C and also by a large number of phenolic compounds present in purple yam tuber (Tamaroh et al., 2018) . Pigment retention percentage is an indication of the overall stability of anthocyanin in juice model systems. Results clearly indicated the influence of storage temperature on anthocyanin degradation. Refrigeration drastically improved pigment stability in the red cabbage anthocyanin. Purple yam anthocyanin shows comparatively high stability than red cabbage anthocyanins at both storage conditions. An abundance of acylated anthocyanins in purple yam may be the reason for this stability. Monomeric anthocyanin content and polymeric percentage are considered as the color quality determinants of natural colorants. Monomeric anthocyanins were reported as an extremely unstable compound that degraded into colorless or brown-color compounds. Different factors such as temperature, pH, oxygen, pigment

concentration, light, coupling of copigments, enzymes and metallic ions influence the rate and extent of monomeric anthocyanin degradation. This degradation of anthocyanins were usually accompanied by an increase in polymeric color percentage (Sinela et al., 2017; Martynenko, 2016; Jiang et al., 2019) . In both purple yam and red cabbage anthocyanin-based juice model systems, refrigerated samples maintained more than 75% of the initial concentration of monomeric anthocyanin over a period of six months. At room temperature purple yam anthocyanin colored juice system maintained about 70% of monomeric anthocyanin and this fact suggests it's applicability in the food industry. Polymeric color % in both juice systems tend to increase over time but refrigeration reduced its extent over time. Purple yam colored juice system stored in cold temperature showed the least development of polymeric color and it can be correlated with extended stability of monomeric anthocyanin. Rodríguez-Saona et al. (1999) also reported the correlation between monomeric anthocyanin degradation and polymeric color development in red radish anthocyanin colored juice model systems . Both red cabbage and purple yam juice system exhibited a high initial % polymeric color (20%). The complex composition of the crude extracts may account for this increase in polymeric color. According to previous reports, anthocyanin degradation under storage tends to follow a first-order reaction kinetics (Concepcion , 2000). Anthocyanin degradation in red cabbage and purple yam also followed first-order reaction kinetics and clearly influenced by the storage temperature. The determination coefficients obtained in this experiment ranged from R^2 = 0.904 to 0.905 showing a good correlation between the anthocyanin concentration, time and storage temperature . The estimated half-lives of monomeric anthocyanin were highest in the case of purple yam juice system (47weeks) while in the case of red cabbage it was calculated as 41 weeks. Half-lives of total anthocyanin content which includes both monomeric and polymeric anthocyanin were even higher in both purple yam and red cabbage (141 weeks for purple yam and 90 weeks for red cabbage under refrigeration). The monomeric half-life of purple yam and red cabbage anthocyanins was found to be higher than other sources of anthocyanin like purple potato, Eggplant peel, Strawberry, Grape, Bilberry and red Plum peel (Reyes, 2007;Hernández et al., 2011). This higher stability could be due to the predominance of acylated anthocyanins in purple yam and red cabbage.

The color attributes of anthocyanin in juice model systems tended to change according to the nature of pigments and storage temperature. Chroma is derived from the* and b* coordinates and indicates saturation of colour (Wrolstad,1993). Under refrigeration purple yam anthocyanin containing juice system maintained the chroma for up to six months without a considerable change while in red cabbage systems chroma reduced up to 10 points. At room temperature change in chroma was drastic in red cabbage anthocyanin containing juice system while purple yam anthocyanin coloured juice system maintained chroma up to 13 weeks without a noticeable change. Lightness (L*) tend to decrease in all systems but the extent was higheratroom temperature. Stability of purple yam anthocyanins in juice model systems could be explained by the presence of the unusually stable monoacylated anthocyanin, Alatamn C. The stability of this particular compound depends on the "intramolecular stacking of sinapic acid and to the chiral self-association of anthocyanin nuclei " (Yoshida et al.,1991).

4. CONCLUSIONS

Absorption spectra and CIELab color coordinates of purple yam and red cabbage extracts suggested that they could be a natural alternative to FD&C Red # 3 in juice model systems. Red cabbage extract imparts a red color with a blue hue to the juice model systems while purple yam produces red color with a yellow hue. Pigment retention percentage was higher in purple yam based

juice systems. Anthocyanin degradation was dependent on storage temperature and refrigeration reduced its rate. At room temperature, the half-life of monomeric anthocyanin in purple yam colored juice was higher than red cabbage anthocyanin colored juice systems. Refrigeration further improved the stability and color characteristics of juice systems. Purple yam anthocyanins showed higher pigment and color stability than red cabbage anthocyanins in juice systems.

5. REFERENCES

[1]. Scotter MJ, (2003) Properties and determinants of synthetics pigments. In: Encyclopedia of Food Sciences and Nutrition (2nd Edition), Elsevier, pp.1556-1567 .

[2]. Dixit S, Khanna SK and Das M. (2013) All India Survey for Analyses of Colors in Sweets and Savories: Exposure Risk in Indian Population. Journal of Food Science 78 (4), 642-647.

[3]. Tsai CF, Kuo CH and Shih D YC. (2015) Determination of 20 synthetic dyes in chili powders and syrup-preserved fruits by liquid chromatography/tandem mass spectrometry. journal of food and drug analysis 23 (1) 453-62

[4]. Bateman B, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald C, Stevenson J. (2004) The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Archives of Disease in Childhood 89(1), 506–511.

[5]. Feingold BF. (1975)Hyperkinesis and learning disabilities linked to artificial food flavors and colors. Am J Nurs75:797-803

[6]. Kobylewski S & Jacobson MF (2012) Toxicology of food dyes, International Journal of Occupational and Environmental Health 18:3, 220-246

[7]. Giusti MM. Rodriguez-Saona LE, Wrolstad RE. (1999) Molar absorptivity and color characteristics of acylated and non-acylatedpelargonidin-based anthocyanins. Journal of Agricultural and Food Chemistry 47(11): 4631–4637.

[8]. He J, Giusti M. (2010) Anthocyanins: natural colorants with health-promoting properties. Annual Review of Food Science and Technology 1 (1): 163–87

[9]. Ongkowijoyo Paulina, Diego A. Luna-Vital, and Elvira Gonzalez de Mejia. (2018) Extraction Techniques and Analysis of Anthocyanins from Food Sources by Mass Spectrometry: An Update Food Chemistry 250: 113–26.

[10]. Wallace TC. andGiusti, MM. (2008) Determination of color, pigment, and phenolic stability in yogurt systems colored with nonacylatedanthocyanins from Berberisboliviana L. as compared to other natural/synthetic colorants. Journal of Food Science, 73(4)

[11]. Brenes Carmen H, David Del Pozo-Insfran and Stephen T Talcott, Stability of CopigmentedAnthocyanins and Ascorbic Acid in a Grape Juice Model System, Journal of Agricultural and Food Chemistry, 2005, 53, 49−56.

[12]. Dyrby M, Westergaard N. and Stapelfeldt, H. (2001) Light and heat sensitivity of red cabbage extract in soft drink model systems Food Chemistry 72(1) ,431–37.

[13]. Henry BS (1996) Chapter 2 In. Natural Food Colorants, 2nd ed., G.A.F. Hendry and J.D. Houghton(2ndEd.), Blackie A&P, Great Britain, , p. 39-78.

[14]. He H, Gao G, Liu L, Hu X, Tang, X, Zhao J and H. Jin.(2015) Identification of Anthocyanins in Chinese Purple Yam (DioscoreaAlata L.) by High Performance Liquid Chromatography - Ion Trap Time of Flight Tandem Mass Spectrometry (LCMS-IT-TOF), ActaHorticulturae, ,1106, 93-100

[15]. Shoyama Yukihiro, Itsuo Nishioka, Water Herath, Shunpei Uemoto,TKunimitsuFujieda and Hiroshi Okubo. (1990) Two AcylatedAnthocyanins From DioscoreaAlataPhytochemistry29 (9): 2999–3001.

[16]. Zhang Junhong, Honglei Tian, Ping Zhan, Fangling Du, AizhenZong, and Tongcheng Xu. (2018) Isolation and Identification of Phenolic Compounds in Chinese Purple Yam and Evaluation of Antioxidant Activity LWT Food Science and Technology 96:161–65.

[17]. Bakowska-Barczak Anna. (2005) AcylatedAnthocyanins As Stable , Natural Food Colorants A Review, Polish Journal of Food and Nutrition Science 14 (2):107–15

[18]. Piccaglia R, Marotti M, Baldoni G. (2002) Factors influencing anthocyanin content in red cabbage (Brassica oleraceavarcapitata Lf rubra (L) Thell). Journal of Science,Food and Agriculture. 82: 1504−1509

[19]. Charron CS, Clevidence BA, Britz SJ, Novotny JA. (2007) Effect of dose size on bioavailability of acylated and nonacylatedanthocyanins from red cabbage (Brassica oleracea L. var. capitata), Journal of Agricultural and Food Chemistry 55: 5354−5362

[20]. Wu X, Ronald L. (2005) Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. Journal of Agricultural and Food Chemistry,53: 2589-2599

[21]. Rodríguez-Saona LE, MM Glusti, and RE Wrolstad. (1999)Color and Pigment Stability of Red Radish and Red-Fleshed Potato Anthocyaninsin Juice Model Systems,Journal of Food Science 64 (3):451–56.

[22]. GiustiMónica and Ronald E. Wrolstad (2001) Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy In. Current Protocols in Food Analytical Chemistry, John Wiley & Sons, pp. 19–31.
[23]. Itle Rachel A and Eileen A. Kabelka. (2009) Correlation between lab color space values and carotenoid content in pumpkins and squash (Cucurbita Spp.). Horticultural Science, 44 (3): 633–37
[24]. AhmadianiNeda, Robbins RJ, Collins TM., and Giusti MM. (2014) Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. Journal of Agricultural and Food Chemistry 62 (30): 7524–31.
[25]. Sigurdson Gregory T, Robbins RJ, Collins TM., and Giusti MM. (2018) Impact of location, type, and the number of glycosidic substitutions on the color expression of o-dihydroxylatedanthocyanidins. Food Chemistry 268: 416–23.
[26]. Moriya Chiemi, Takahiro Hosoya, SayuriAgawa, Yasumasa Sugiyama, IkukoKozone, Kazuo Shin-ya, Norihiko Terahara& Shigenori Kumazawa (2015) New acylatedanthocyanins from purple yam and their antioxidant activity. Bioscience, Biotechnology and Biochemistry 79 (9):1484–92.
[27]. TamarohSiti, Sri Raharjo, Agnes Murdiati, and Sri Anggrahini. (2018) Total phenolic content and antioxidant activity of anthocyanin extract from purple yam (DioscoreaAlata L.) Flour Using Different Solvents. Pakistan Journal of Nutrition 17 (6):260–67
[28]. Sinela André, NadirahRawat, Christian Mertz, NawelAchir, Hélène Fulcrand, and Manuel Dornier. (2017) Anthocyanins degradation during storage of Hibiscus sabdariffa extract and evolution of its degradation products. Food Chemistry 214 :234–41.
[29]. Martynenko Alex, Yougui Chen. (2016) Degradation kinetics of total anthocyanins and formation of polymeric color in blueberry hydrothermodynamic (HTD) processing. Journal of Food Engineering 171: 44-51
[30]. Jiang Tian, Ying Mao, Lushan Sui, Ning Yang, Shuyi Li, Zhenzhou Zhu, , ChengtaoWangb,ShengYinc, JingrenHea, YiHea. (2019) Degradation of anthocyanins and polymeric color formation during heat treatment of purple sweet potato extract at different PH, Food Chemistry. 274 : 460–70.
[31]. Concepcion Romero and Bakker Johanna. (2000) Effect of Storage Temperature and Pyruvate on Kinetics of Anthocyanin Degradation, Vitisin A Derivative Formation, and Color Characteristics of Model Solutions. Journal of Agricultural and Food Chemistry,48 (6): 2135–41.
[32]. ReyesLFand Cisneros-Zevallos L. (2007) Degradation kinetics and colour of anthocyanins in aqueous extracts of purple- and red-flesh potatoes (Solanumtuberosum L.). Food Chemistry 100 (3): 885–894.
[33]. Hernández-Herrero, José Antonio, and María José Frutos. (2011) Degradation kinetics of pigment, color and stability of the antioxidant capacity in juice model systems from six anthocyanin sources. International Journal of Food Science and Technology, 46 (12) : 2550–57.
[34]. Wrolstad, R. E. (1993). Color and pigment analyses in fruit products. Agricultural Experiment Station Oregon State University Station Bulletin, 624, 1–17
[35]. Yoshida K, Kondo T, Kameda K, Kawakishi S, LubagAjm, Mendoza Emt AndGoto T. (1991) Structures of alatanins A, B, and C isolated from purple yam Dioscoreaalata. Tetrahedron Letters ,32: 5575–5578.