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Anthocyanins from mulberry (*Morus rubra)* fruits as potential natural colour additives in yoghurt

Robert Byamukama[1]*, Moses Andima[1,2], Angella Mbabazi[1] and Bernard T. Kiremire[1]

[1]Department of Chemistry, Makerere University, P. O. Box 7062, Kampala, Uganda.
[2]Department of Chemistry, Busitema University, P. O. Box 236, Tororo, Uganda.

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Colouring potential of anthocyanins from whole fruit juice of mulberry (*Morus rubra)* was studied in yoghurt. Whole fruit juice from *M. rubra* rich in non-acylated anthocyanins was incorporated into plain yoghurt (100 g) at increasing concentration levels of the juice; 10, 20, 25, 30, 40 and 50 mg cyanidin-3-glucopyranoside equivalents (cy-3-glu eqv) and stored under refrigerated condition (< 8°C) for two weeks. Colour properties, pigment and colour stability and degradation kinetics were studied using a UV-Vis spectrophotometer (UV-1700 CE Shimadzu, Japan).Yoghurt coloured with mulberry anthocyanins between 25 to 40 mg cy-3-glu eqv concentration levels of anthocyanins produced a colour which was very much comparable to commercial brand strawberry yoghurt coloured with 20 mg FD & C red No. 3 in 100 g of yoghurt. Pigment and colour stabilities of the anthocyanins increased with increasing concentration of anthocyanins added to yoghurt. The tendency to polymerise decreased with increasing concentration of the pigments added to yoghurt. The degradation of the anthocyanins was fitted to first-order reaction kinetics. Moderate concentration levels (25 to 40 mg cy-3-glu eqv in 100 g of yoghurt) of mulberry anthocyanins were found to be ideal to colour yoghurt.

Key words: Mulberries, anthocyanins, cyanidin-3-glucopyranoside (cy-3-glu), natural colourants, colour stability, yoghurt.

INTRODUCTION

One of the vital constituents of some foods and beverages is colour. In addition to other functions (Mendi et al., 2000), colour plays a very important role in the acceptability of some foods by many consumers (Giusti and Wrolstad, 2003). In practice, most manufacturers tend to colour products which have dull colours and look unappealing to most consumers. Synthetic colourants have often been used in attempts to colour some foods and beverages (Delgados-Vargas and Paredes-López, 2003). However, the demand for foods with synthetic colours is diminishing drastically due to associated health problems and legislative action against some of them (Cevallos-Casals and Cisneros-Zevallos, 2004; Markakis, 1982).

Anthocyanins have been of great interest as alternatives to synthetic colourants due to their bright colours and associated health benefits (Cevallos-Casals and Cisneros-Zevallos, 2004; Fan et al., 2008). They are considered to be safe because they have been consumed for centuries in fruits, and vegetables without

*Corresponding author. E-mail: rbyamukama@cns.mak.ac.ug, Tel: +256392548585.

any health risks (Bridle and Timberlake, 1997). The application of anthocyanins as food colourants faces some limitations such as low stability to several processing, formulation and storage conditions. Despite the drawback, highly acylated anthocyanins possess high colour stability in some food matrices (Giusti and Wrolstad, 2003; Kirca et al., 2006). Nevertheless, Wallace and Giusti (2008) reported that whole fruit extracts containing non-acylated anthocyanins from *Berberis boliviana* L. showed improved colour and pigment stability when incorporated in yoghurt. This information led us to believe that whole fruit juice extracts from mulberries (*Morus rubra*) could serve as an appropriate colourant and nutraceautical in yoghurt.

Mulberries (*M. rubra*) are a rich source of anthocyanins, with fully ripe fruits yielding between 2.53 to 8.30 mg cy-3-glu/gfresh weight of anthocyanins (Özgen et al., 2009). Other studies reported the content of anthocyanins to vary from 0.59 mg/ g to 10.90 mg/ g fresh weight infrom different cultivars of mulberries (Liu et al., 2004).The fruits possess many health benefits such asantithrombotic, antioxidant, antimicrobial, anti-inflammatory, and neuroprotective properties(Aramwit et al., 2010). In recent years, mulberry anthocyanins have in many occasions been applied in baked foods, and confectioneries (Wu et al., 2011). In Turkey, they are used to make special important traditional products such as mulberry pekmez, mulberry pestil, and mulberry kome (Ercisli and Orchan, 2007). The anthocyanins are stable at low temperatures and in the dark (Aramwit et al., 2010). In this study, we decided to investigate whether anthocyanins from mulberries (*M. rubra*) could be used as potential colour additives in yoghurt since yoghurt has a low pH and it is stored under refrigerated conditions.

MATERIALS AND METHODS

Plant materials and yoghurt

Ripe fruits of mulberries (*M. rubra)* were collected from Masaka District in Uganda and stored in a laboratory freezer before use. Plain yoghurt (1.5% fat) at the point of adding colours and commercial brand strawberry flavoured yoghurt were kindly provided by fresh dairy factory (Sameer Agriculture and Livestock (U) ltd, Plot 49-53/55 fifth street, Industrial Area, Kampala, Uganda).

Chemicals, reagents and equipment

Analytical grade methanol, potassium chloride, sodium acetate, and potassium metabisulphite used in this study were purchased from BDH laboratory suppliers (U) ltd. A UV-Vis spectrophotometer (UV-1700 CE Shimadzu) was used for spectrophotometric measurements using 1 cm path length disposable cuvette tubes.

Preparation of whole-fruit juice concentrate

Frozen fruits of mulberry were allowed to defrost and the fruits were pasteurised immediately to denature enzymes such as polyphenol oxidase (PPO) which causes browning of anthocyanins (Wang and Xu, 2007). The fruits were then blended in a laboratory blender (Waring 700S 1 L, Lab depot, Inc.) and the juice was filtered through a clean piece of nylon clothe. The juice was centrifuged at 4000 rpm and the supernatant was concentrated in a vacuum rotary evaporator at 35°C to 15% brix. The concentrate was kept below 4°C in a freezer until analysis.

High-performance liquid chromatography (HPLC) analysis

To confirm the anthocyanins in *M. rubra,* high performance liquid chromatography (Shimadzu, Columbia, Md.,U.S.A) was performed on a Hypercil gold^{TM} reverse phase 250 x 4.6 mm symmetry, 5 µm size C18 column (Thermo Fisher Scientific Inc.) equipped with a photo array detector (PAD). Separation of the anthocyanins was achieved using a linear gradient. Solvent A was HCOOH:H_2O; 1:19, Solvent B was HCOOH:H_2O:MeOH; 1:9:10. The elution profile was 0 to 23 min, 10 to 100% B, 0 to 5 min 10% B, 5 to 23 min, 100% B, 23 to 28 min, 100% B 28 to 29 min, 10% B at a flow rate of 0.75 ml min^{-1} and the injection volume was 15 µl. UV-Vis spectral data was obtained from 250 to 560 nm (Wallace and Giusti, 2008).

Colouring yoghurt with the juice extract

100 g portions of plain yoghurt at the point of adding colour were coloured with the whole fruit juice extract of mulberries at increasing concentration levels of the juice (10, 20, 25, 30, 40, and 50 mg Cy-3-glu eqv) in 100 g of yoghurt. The amount of the juice extract for each portion of yoghurt was chosen in order to study the effect of increasing pigment concentration on the colour of yoghurt and the stability of the pigment in yoghurt (Wallace and Giusti, 2008). Plain yoghurt was manually mixed gently and thoroughly with the juice concentrate to ensure uniformity and avoid syneresis of the set yoghurt. Yoghurt samples were prepared in triplicate at each colouring level. The coloured yoghurt samples were packed in McCartney bottles (50 ml, 144 bottles in total, 3 at each colouring level) and stored in a laboratory refrigerator (< 8°C)for two weeks during which, colour properties, pigment concentration and pH were measured at regular time intervals (Wallace and Giusti, 2008).

Extraction of anthocyanins from the yoghurt matrix

Anthocyanins were recovered from the coloured yoghurt matrix using the method adopted by (Wallace and Giusti, 2008). Coloured yoghurt samples (100 g) at each concentration level, were thoroughly mixed with methanol acidified with hydrochloric acid (99.9%, 0.1%, 30 ml) for 5 min and centrifuged at 4000 rpm for 15 min. The extraction procedure was carried out twice on each sample to ensure maximum recovery of anthocyanins from the yoghurt matrix. The supernatant in each case was carefully filtered and concentrated to a known volume at 35°C with a rotary evaporator. The concentrates were used for spectrophotometric analysis in triplicate. To determine the recovery rates, juice containing anthocyanins (20 mg cy-3-glu eqv) were added to plain yoghurt (100 g) and extracted similarly with acidified methanol. The recovery rate was expressed as a percentage of the recovered amount in the total initial amount added to yoghurt Equation (1). This procedure was repeated six times to determine the extraction efficiency.

$$Recovery\ rate = \frac{amount\ recovered}{Original\ amount\ added\ to\ yoghurt}\ x\ 100 \quad (1)$$

Determination of anthocyanin content

The content of total anthocyanins in the coloured samples

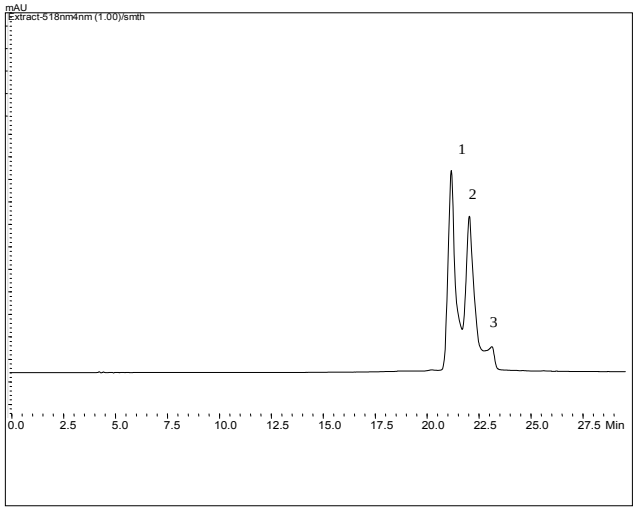

Figure 1. HPLC-PAD chromatogram of anthocyanins in M. rubra fruit juice extract recorded at 520 nm. Cyanidin-3-O-glucopyranoside (peak 1), cyanidin-3-O-rutinoside (peak 2), pelagornidin 3-O-glucopyranoside (peak 3).

remaining at any time was determined using the pH-differential method described by Giusti and Wrolstad (2001).

Evaluation of pigment and colour stability

To evaluate pigment and colour stability, the extent of degradation of mulberry anthocyanin pigments and colour in yoghurt was determined by calculating the percent change in absorbance using Equation (2) (Katsaboxakis et al., 1998).

$$\text{Pigment and colour stability} = \frac{A_t}{A_0} x100 \quad (2)$$

Where A_t is the absorbance of anthocyanins at any time *t, A_0* is the initial absorbance of anthocyanins added to yoghurt at each concentration level. Any variations in colour were confirmed by visual inspection (Brenes et al., 2005; Torskangerpoll and Andersen, 2005). Visual inspection is the commonest method used according to Sameer Agriculture and livestock (U) ltd factory manual basing on the fact that consumers rely on the visual colour to product selection.

Measurement of polymeric colour

The extent of anthocyanin polymerisation in yoghurt matrix was determined using the bisulphite bleaching method (Giusti and Wrolstad, 2001).

Kinetic calculations and statistical analysis

In order to evaluate the degradation kinetics of mulberry anthocyanins in yoghurt, anthocyanin concentration was plotted against time and linear regression analysis was used to determine the adequacy of the anthocyanin degradation kinetic model using Minitab (Mtb14) software. The degradation constant (*k*) was determined from the 1st derivative of the curves plotted using Equation (4) and the half-lives ($t_{1/2}$) at each concentration level were calculated from Equation (5) (Kirca et al., 2006; Wallace and Giusti, 2008).

$$ln\frac{C_t}{C_0} = -kt \quad (4)$$

$$t_{1/2} = \frac{In2}{k} \quad (5)$$

Where C_t is the concentration of anthocyanins at time *t*, C_o is the initial concentration of anthocyanins and *t* is the storage time (shelf life) and *k* is the degradation constant.

Graphs and statistical analysis

Summary statistics (mean and standard deviation), graphs and linear regression analysis were obtained using Microsoft® excel 2007 (Microsoft corp., 2013) as well as Minitab (Mtb16. Exe) software (Minitab® Inc.). Results for colour, pigment stability and degree of polymerisation were subjected to regression analysis and analysis of variance (ANOVA) with an alpha P<0.05 acceptance level (Kirca et al., 2006; Wallace and Giusti, 2008).

RESULTS AND DISCUSSION

Confirmation of the anthocyanins in the *M. rubra* extract

Two major anthocyanins (Cyanidin-3-*O*-glucopyranoside and cyanidin-3-*O*-rutinoside) and two minor anthocyanins (Pelargonidin 3-*O*-glucopyranoside and pelargonidin 3-*O*-rutinoside) have been identified to be present in mulberry fruits (Qin et al., 2010). In this study, the identities of these anthocyanins were confirmed by comparing their order of elution from a C-18 column (Figure 1). Cyanidin-

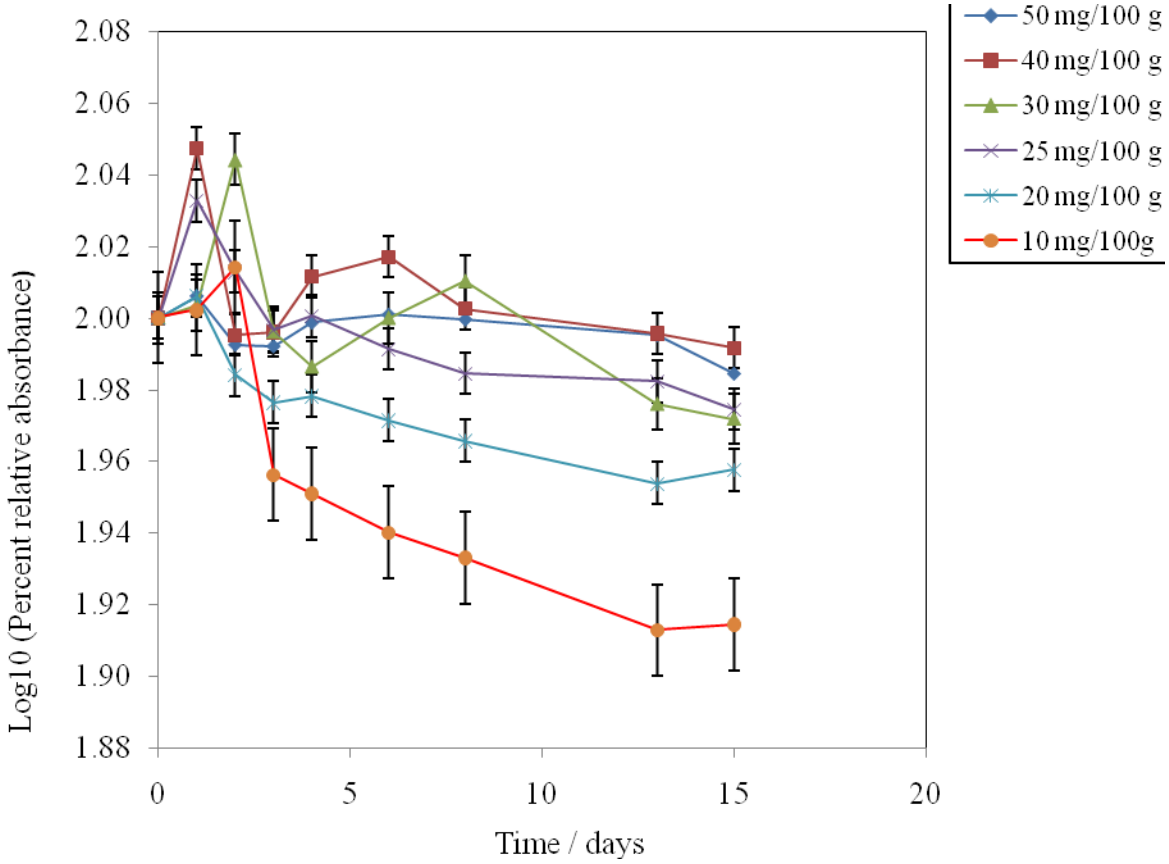

Figure 2. Variation of percent relative absorbance at all concentration levels of anthocyanins; 1020, 25, 30, 40 and 50 mg cyanidin-3-glucopyranoside equivalents in 100 g of yoghurtin 14 days.

3-*O*-glucopyranoside (peak 1) and cyanidin-3-*O*-rutinoside (peak 2) were the major anthocyanins with only one minor anthocyanin (peak 3) (Figure 1). The average content of monomeric anthocyanins in *M. rubra* was 7.30 mg/g fresh weight (STDEV = 0.01, n = 3) as determined by pH differential method (Giusti and Wrolstad, 2001). This concentration was within the same range as reported by Özgen et al. (2009).

Pigment stability

In this study, pigment stability was evaluated in terms of percent relative absorbance. The anthocyanin recovery rate was calculated to be 96.55% (n= 6, STDEV 1.04). Generally, percent relative absorbance increased in the first two days of the investigations and, thereafter, it decreased at all concentration levels (Figure 2). Stabilisation of anthocyanin molecules as they associate with constituents of the yoghurt matrix such as sugars, stabilizers and milk fats could lead to the increased stability in the first two days (Jing and Giusti, 2005; Wrolstad et al., 1990). However, at 30 and 40 mg cy-3-glu eqv of the juice extract in 100g of yoghurt, percent relative absorbance increased gradually between the fourth and eighth days before decreasing, up to the end of the second week (Figure 2).

Concentration levels of 30-50 mg cy-3-glu eqv of mulberry juice containing anthocyanins added to yoghurt showed increased pigment stability ($P > 0.05$) than at lower concentration levels (10 to 25 mg cy-3-glu eqv) ($P < 0.05$) (Figure 2). The results indicated that pigment stability increased with increasing concentration level of the pigments added to yoghurt. Nevertheless, concentration level of 40 mg cy-3-glu eqv in 100 g of yoghurt was the best ($P = 0.248$) because it gave the highest percent relative absorbance than at 30 and 50 mg concentration levels ($P = 0.079$ and 0.099) respectively.

Increased pigment stability at high concentration is attributed to anthocyanin stabilisation through intramolecular association between anthocyanins and intermolecular association with other flavonoids, amino acids, organic acids, nucleotides, polysaccharides or metals that could be present in the matrix (Castañeda-Ovando et al., 2009). These stabilisation mechanisms increase with increasing concentration. At lower concentration levels, intermolecular association

Table 1. Variation of λvis-max and absorbance with storage time at all anthocyanins concentration levels;10,20, 25, 30, 40 and 50 mg cyanidin-3-glucopyranoside equivalents in 100 g of yoghurt (n = 3, P<0.05).

Time (days)	10 mg cy-3-glu eqv			20 mg cy-3-glu eqv			25 mg cy-3-glu eqv			30 mg cy-3-glu eqv			40 mg cy-3-glu eqv			50 mg cy-3-glu eqv		
	λvis-max (nm)	Abs (Au)	Monomeric anthocyanin (cy-3-glu eqv	λvis-max (nm)	Abs (Au)	Monomeric anthocyanin (cy-3-glu eqv	λvis-max (nm)	Abs (Au)	monomeric anthocyanin(cy-3-glu eqv)	λvis-max (nm)	Abs (Au)	Monomeric anthocyanin (cy-3-glu eqv)	λvis-max (nm)	Abs (Au)	Monomeric anthocyanin (cy-3-glu eqv)	λvis-max (nm)	Abs (Au)	monomeric anthocyanin (cy-3-glu eqv)
0	510.8	0.240	10.02± 0.01	510.8	0.48	20.04± 0.00	510.8	0.60	25.06± 0.02	511.0	0.36	30.00± 0.01	511.0	0.48	40.01± 0.02	511.0	0.60	50.01±0.01
1	511.8	0.241	10.05± 2.3	511.8	0.49	20.32± 0.03	511.0	0.65	26.94± 0.22	511.0	0.36	30.23± 0.72	511.0	0.53	44.59± 0.23	511.0	0.61	50.72± 0.12
2	511.0	0.248	10.35± 0.11	511.0	0.46	19.32± 0.24	510.5	0.62	25.74± 0.19	511.0	0.40	33.20± 0.60	511.0	0.47	39.57± 0.08	511.0	0.59	49.16± 2.21
3	511.0	0.217	9.06± 0.07	511.0	0.45	18.98± 0.18	510.7	0.59	24.79± 0.13	511.0	0.36	29.72± 0.49	511.0	0.47	39.64± 1.65	511.0	0.59	49.09± 0.24
4	519.3	0.214	8.95± 3.0	521.0	0.46	19.06± 0.30	520.0	0.60	25.03± 0.32	511.5	0.35	29.06± 0.23	511.5	0.49	41.09± 0.13	511.5	0.60	49.83± 0.13
6	511.0	0.209	8.73± 0.17	508.0	0.45	18.76± 0.29	508.0	0.59	24.5± 0.44	511.0	0.36	30.00± 0.27	511.0	0.50	41.61± 0.50	511.0	0.60	50.11± 1.40
8	511.0	0.206	8.59± 0.10	507.8	0.44	18.51± 0.11	510.0	0.58	24.13± 0.28	511.0	0.37	30.72± 0.56	511.0	0.48	40.26± 0.59	511.0	0.60	49.96± 0.14
13	511.0	0.196	8.19± 0.09	512.5	0.43	18.02± 0.05	512.0	0.58	24.01± 0.20	511.0	0.34	28.39± 0.31	511.0	0.47	39.60± 0.03	511.0	0.59	49.47± 1.06
15	512.5	0.197	8.22± 0.15	511.0	0.44	18.18± 0.94	512.8	0.56	23.56± 0.48	511.0	0.34	28.11± 0.05	510.5	0.47	39.24± 1.05	510.5	0.58	48.27± 0.74

Monomeric anthocyanin recorded as mean±Stdev (n= 3, P <0.05).

predominates but at higher concentration levels, both intra and intermolecular associations are present (Gauche et al., 2010; Nielsen et al., 1993). In similar studies, Giusti and Wrolstad (2003) noted that increased anthocyanin concentration in food systems promoted higher colour stability. Likewise, pigment and colour stability of strawberry syrup increased greatly by increasing the concentration of anthocyanins in the syrup (Cavalcanti et al., 2011).

Despite the high stability of mulberry anthocyanins in this study, some amount of anthocyanins added to yoghurt was lost in the two weeks study at all concentration levels. For example, on the last day, the percent relative absorbencies of anthocyanins at each concentration level were 96.5, 98.1, 93.7, 94.3, 90.7 and 82.1% at 50, 40, 30, 25, 20 and 10 mg cy-3-glu eqv in 100 g of yoghurt respectively (Figure 2). Polymerisation of monomeric anthocyanins with other phenolic compounds and degradation by endogenous or exogenous enzymescan cause gradual loss of monomeric anthocyanins (Brownmiller et al., 2008; Ścibisz et al., 2012; Skrede et al., 2000). Different authors cited by Ścibisz et al. (2012) established that lactic acid culture which is added to yoghurt as a starter produces hydrogen peroxide which accelerates anthocyanin degradation or the lactic acid culture probably produces β-glucosidases which converts anthocyanins to their more unstable aglycone forms.It is clear that one or more of these enzymes or other factors could have caused some gradual degradation of anthocyanins added to yoghurt in this study.

Colour stability

In addition to using percent relative absorbance as a measure of pigment and colour stability, variations in colour hue of mulberry anthocyanins described as absorption spectra, were evaluated at the storage temperature (< 8°C), pH 3.4, at all concentration levels of anthocyanins that were added to yoghurt. On average, the visible λ_{max} for the pigments at all concentration levels was 511.7 nm over the 2 weeks period (Table 1). The absorbance values at visible maximum absorption wavelength decreased gradually with time at all concentration levels as the pigments stayed longer in yoghurt (Table 1). However, the decreases in absorbencies with time at each concentration level were small, suggesting high colour stability, which corresponds to high pigment stability.

An increase in the maximum absorption wavelength (bathochromic shift, $\Delta\lambda_{Vis\text{-}max}$ from 511 to 520 nm) and absorbance (hyperchromic effect, $\Delta\lambda_{Vis\text{-}max}$) were observed on the fourth day at 10-

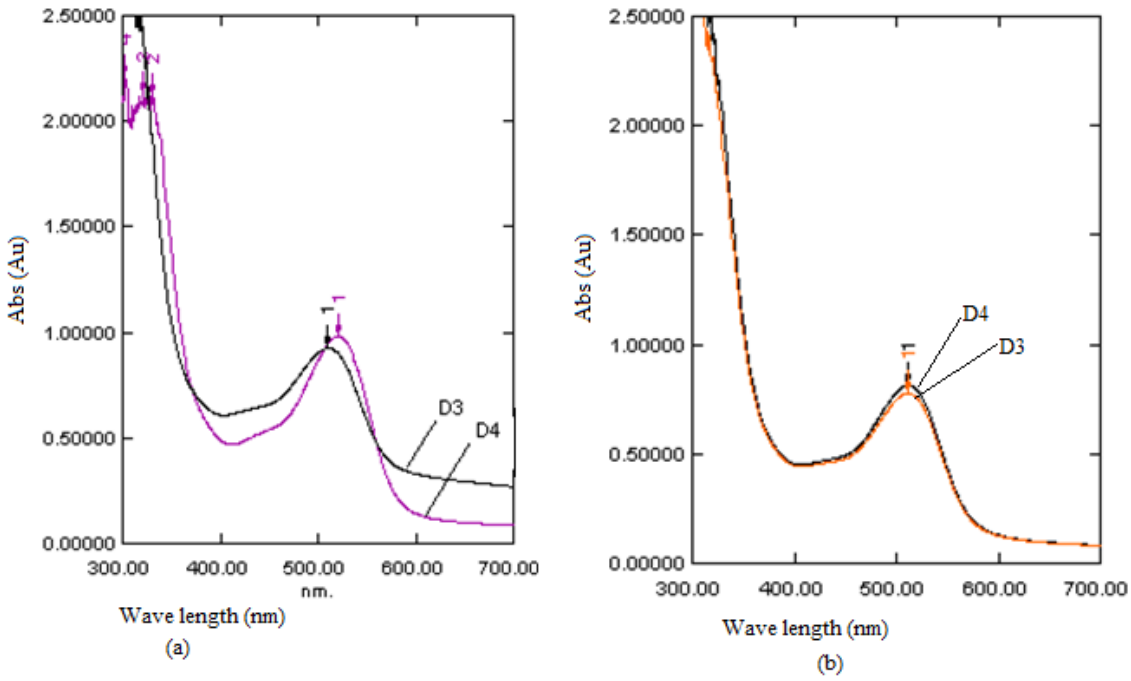

Figure 3. Comparison of absorption spectral pattern of mulberry anthocyanins; (a) 25mg cy-3-glu eqv/100 g and (b) 40 mg cy-3-glu eqv/100 g concentration levels on the third (D3) and fourth (D4) days (n = 3).

25 mg/100 g concentration levels (Table 1). The bathochromic effect was negligibly small between 30-50 mg/100 g concentration levels. For example at 25 mg cy-3-glu concentration level, there was a bathochromic shift from 511 to 521.0 nm between the third and fourth days whereas the bathochromic shift was only 0.5 nm from the third to the fourth day at 40 mg/100 g concentration level (Table 1, Figure 3).

The observed bathochromic and hyperchromic effects at 10 to 25 mg cy-3-glu concentration levels could be attributed to intermolecular association with other polyphenols and other flavonoids (Heredia et al., 1998). At lower concentration levels, intermolecular association of anthocyanins with polyphenols and other flavonoids are stronger than intramolecular associations leading to the observed bathochromic shift and hyperchromic effects (Gauche et al., 2010; Nielsen et al., 1993). Plant constituents such as cell wall and vacuolar components in the mulberry whole fruit juice could have associated with the anthocyanin pigments. Recent studies showed that interaction of plant constituents such as hydroxycinnamic acids in canned strawberries with nonacylated anthocyanins enhanced the colour stability of anthocyanins (Kammerer et al., 2007). Various components in the yoghurt matrix such as added sugar, fats, and stabilizers could have associated with anthocyanins to increase their colour stability (Jing and Giusti, 2005; Wrolstad et al., 1990). On the other hand, under refrigerated conditions, anthocyanins have reduced reaction rates, which could enhance the colour stability of the pigments added to yoghurt.

The variations in colour hue of the yoghurt coloured with mulberry anthocyanins were visually confirmed by comparing with commercial brand strawberry flavoured yoghurt (containing 20 mg FD & C red No. 3 in 100 g of yoghurt) and plain yoghurt as controls (Brenes et al., 2005; Torskangerpoll and Andersen, 2005). Between 25 to 40 mg cy-3-glu eqv in 100 g of yoghurt, the anthocyanins imparted a bright pink colour similar to that of commercial brand strawberry flavoured yoghurt. On the other hand, at 20 mg cy-3-glu eqv in 100 g of yoghurt, a dull/faint pink colour was imparted to yoghurt while at 10 mg cy-3-glu eqv in 100 g of yoghurt, a faded pink colour was imparted to the yoghurt. At 50 mg cy-3-glu eqv in 100 g of yoghurt, the pigments mixed with cream plain yoghurt, producing a colour close to red, which was rather unappealing. It is clear from these results, that concentration levels ranging from 25-40 mg cy-3-glu equivalents in100 g of yoghurt would be ideal for imparting an appealing colour to yoghurt.

Polymeric colour analysis

Polymeric colour was measured to determine the level of anthocyanin pigment polymerisation on addition to yoghurt matrix at all concentration levels. The results indicated that, there was a general increase in percent polymeric colour up to about the sixth day followed by a gradual decrease after the eighth day of storage at all

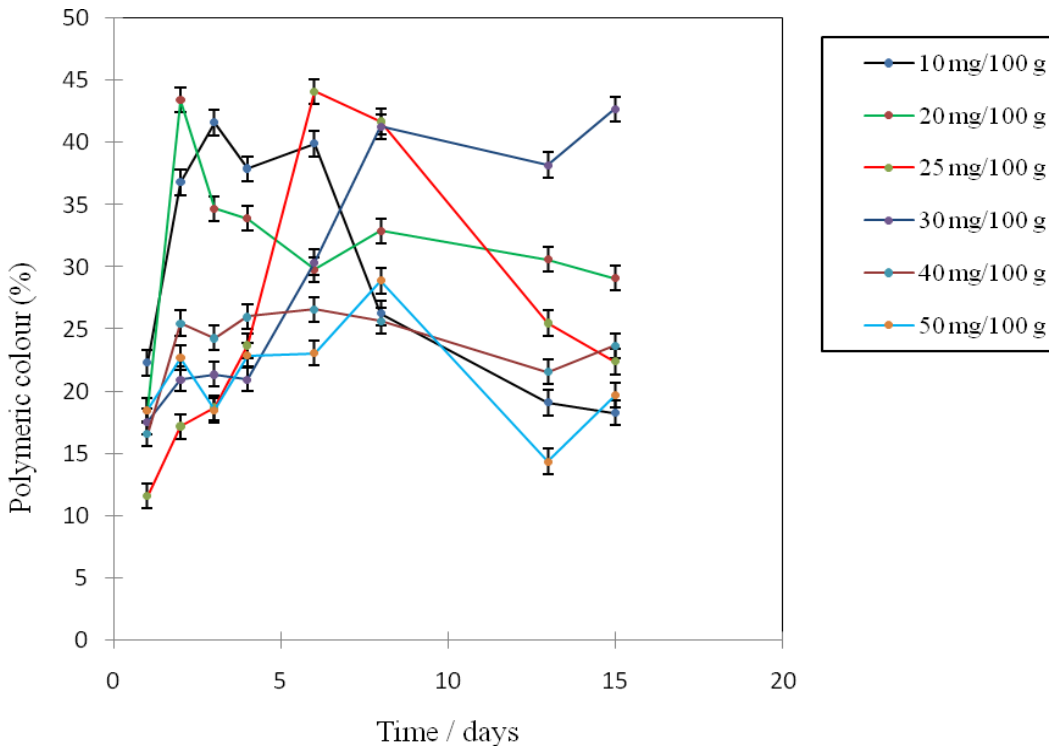

Figure 4. Variation of polymeric colour with time at all concentrations of anthocyanins;10, 20, 25, 30, 40 and 50 mg cyanidin-3-glucopyranoside equivalents in 100 g of yoghurt (n = 3).

concentration levels (Figure 4). The extent of anthocyanin polymerisation was higher at 10, 20, 25 and 30 mg Cy-3-glu eqv ($P < 0.05$) than at higher concentration levels (40 and 50 mg Cy-3-glu eqv) ($P > 0.05$) in 100 g of yoghurt (Figure 4). It was again observed that on the eighth day, concentration 40 mg had the lowest percentage of polymerisation hence more stable anthocyanins. This again supports the trend observed in pigment and colour stability at concentration 40 mg in Figure 2.

Anthocyanins that polymerize may absorb at the maximum wave length of absorption in the visible range (in this case at 511 to 520 nm). This means that they maintain a uniform and stable colour over time (Wallace and Giusti, 2008). However, large polymers tend to precipitate resulting in a decrease in polymeric colour. The increase in polymeric colour in the first six days was therefore due to increase in absorption by polymerised anthocyanins. As the pigments stayed longer in the yoghurt matrix, they could have formed high molecular weight polymers with other polyphenols which precipitated out leading to a drop in polymeric colour and consequently leading to decrease in colour stability.

Degradation kinetics

The degradation of anthocyanins from *M. rubra* in yoghurt matrix was fitted to first-order kinetics. The rates of degradation at lower concentration levels (10 to 25 mg) were higher than at higher concentration levels (30 to 50 mg) (Table 2) which further supports the variation in pigment and colour stability. The half-life of the pigments increased with increasing concentration level suggesting that the pigments were more stable at higher concentration levels than at lower concentration levels (Table 2).

Linear regression analysis was used to determine the adequacy of the degradation kinetic model at alpha (α) < 0.05 acceptance level which indicates that the concentration of the anthocyanins decreased linearly with time (Figure 5). For example, the degradation equation at 20 mg cy-3-glu eqv concentration level was $lnC = -0.00691t + 2.985$ ($P < 0.05$).

A good regression was obtained between concentration and time at 10-25 mg cy-3-glu eqv concentration levels ($P < 0.05$) (Table 2). However other factors in addition to concentration tend to influence the stability of the pigments added to yoghurt between 30-50 mg cy-glu eqv concentration levels ($P > 0.05$) (Table 2). Intermolecular and intramolecular associations are evident at higher concentration levels (Nielsen et al., 1993).Whole fruit juice of mulberries used in this study contains cell wall and vacuolar components which could have exerted protective effect on the pigments at higher concentration levels (Kammerer et al., 2007).

Table 2. Degradation kinetic and linear regression parameters.

Concentration levels (mg cy-3-glu in 100 g)	k (day^{-1})	$t_{1/2}$ (days)	Regression coefficients (R^2)(%)	P > F
10	1.48 x 10^{-2}	46.7	76.9	0.002
20	6.9 x 10^{-3}	100.3	80.2	0.001
25	6.18 x 10^{-3}	112.7	76.9	0.008
30	5.0 x 10^{-3}	138.6	37.6	0.079
40	3.0 x 10^{-3}	231.05	18.5	0.248
50	1.0 x 10^{-3}	693.1	34	0.099

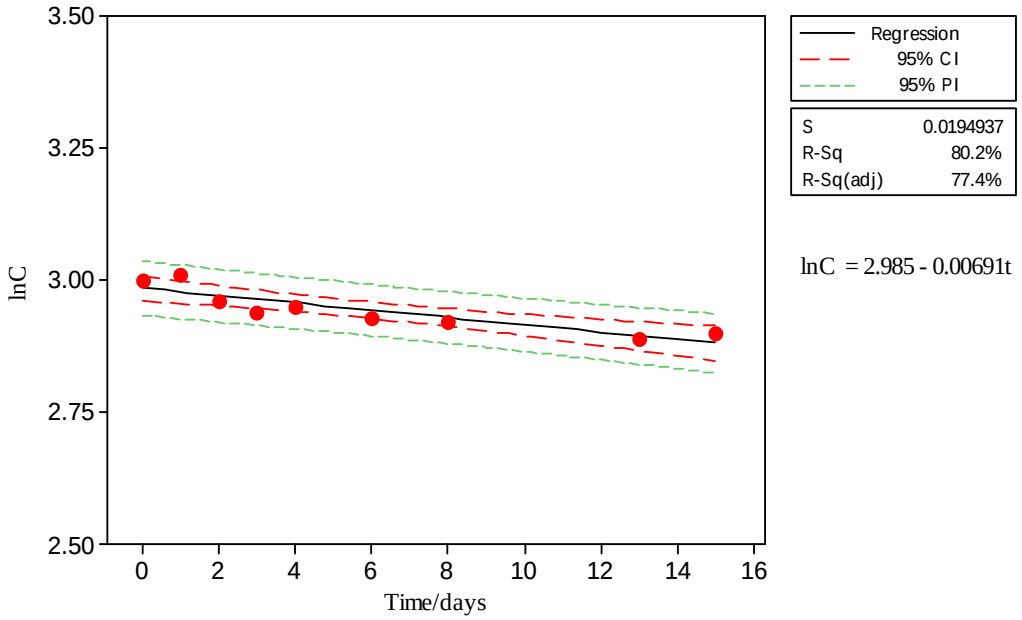

Figure 5. Linear regression predictions of degradation kinetic model at 20 mg cy-3-glu eqv in100 g of yoghurt concentration levels.

Conclusions

Addition of juice from whole fruit of mulberry (25-40 mg cy-3-eqv) rich in anthocyanins to plain yoghurt (100 g) achieved a good colour, matching with that of commercial brand strawberry flavoured yoghurt which was coloured using erythrosine (FD & C Red No. 3, 20 mg in 100 g of yoghurt). Colour and pigment stabilities of the anthocyanin pigments were very high between 25-40 mg cy-3-eqv in 100 g concentration levels. At the same time, the tendency of the pigments to polymerise was very low between 25-40 mg cy-3-glu concentration levels. The half-life for the pigments wasvery high compared to the shelf-life of commercial brand strawberry yoghurt indicating that, anthocyanins from mulberries are very stable when added to yoghurt. Moderate concentration levels (25-40 mg cy-3-glu eqv in 100 g of yoghurt) of mulberry anthocyanins would be ideal to colour yoghurt.

Conflict of Interest

The authors have not declared any conflict of interest.

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