

*Full Length Research Paper*

# The effects of temperature and pH on stability of anthocyanins from red sorghum (*Sorghum bicolor*) bran

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Anthocyanin's have been suggested as promising dietary compounds with an important role in human health. This study focuses on the effect of temperature and pH on stability of anthocyanins from red sorghum bran. The sorghum anthocyanins were found to be stable at 0°C even when exposed to light and dark conditions. The anthocyanins extracted using acidified methanol showed higher stability than methanol extracts. The study on anthocyanin stability over the pH range (1.1 to 10.5) during 20 days storage was conducted on sorghum anthocyanins compared to methanol and acidified methanol extracts afforded higher colour intensity and higher or similar stability throughout the whole pH range. At pH 8.9, the colour intensity of sorghum anthocyanins was even higher than at the lowest pH values. The sorghum 3-deoxyanthocyanins were a rare class of plant pigments, and were stable to light, heat and change in pH. The use of sorghum anthocyanins as food colourants in slightly alkaline products (bakery, milk, egg, etc.) can therefore be considered.

**Key words:** Sorghum, bran, anthocyanins, temperature, pH stability.

## INTRODUCTION

Functional foods and nutraceuticals are rapidly being integrated into the corporate mainstream and increasing being accepted by the public due to a steady demand from consumers for healthier foods and products. Anthocyanins are of particular interest to the food colourant industry due to their ability to impart vibrant colours. However, due to a rising demand for economical sources of natural, stable pigments, there is increased interest in alternative sources of anthocyanins, especially cereals, including rice (Ryu et al., 1998; Hu et al., 2003), wheat (Abdel-Aal and Hucl, 1999, 2003) and sorghums (Nip and Burns, 1969; Gous, 1989).

Factors that affect the stability of anthocyanins include

structure, pH, temperature, light, Co pigment, etc. (Rodriguez-Saona et al., 1999). The type of anthocyanidin, its glycos of anthocyanidin, its glycosylation pattern, and its acylation with aromatic and/or aliphatic acids have an important effect on anthocaynin stability (Stintzing et al., 2002; Shi et al., 1994; Baublis et al., 1994; Guisti et al., 1999). Anthocyanins acylated with aromatic acids showed higher storage stability as compared to nonacylated counter parts (Inami et al., 1996). It was shown that the concentration of polymeric pigments increases with temperature and storage time (Garcia-Viguera et al., 1998).

In sorghum, the most common anthocyanin types are the 3-deoxyanthocaynidins and their derivatives. These anthocyanins, which include luteolinidin and apigeninidin, are not commonly found in higher plants (Clifford, 2000).

The objective of this research was to investigate the

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stability of red sorghum anthocyanins at different temperature and pH.

## MATERIALS AND METHODS

### Samples

The bran of *Sorghum bicolor* (L.) red sorghum was collected from farmers' field in Tamil Nadu and was stored at -20°C.

### Sample extraction

Two extraction solvents of 1% HCl in methanol and methanol were used for extraction procedure which involved the addition of 10 ml solvent to 0.5 g sample in 50 ml centrifuge tubes and shaking the samples for 2 h at low speed in an orbit shaker (Neolab). Samples were then stored at -20°C in the dark overnight to allow for maximum diffusion of phenolics from the cellular matrix. Samples were then equilibrated to room temperature and centrifuged at 7000 g for 10 min and taken for analysis. Residues were rinsed with two additional 10 ml volumes of solvent with shaking for 5 min, centrifuging at 7000 g for 10 min, and taken for analysis. The three aliquots were mixed and stored at -20°C in the dark until biochemical analysis.

### Effect of temperature on pigment stability

Amber glass bottles (50 ml) provided with a self-sealing rubber cap and aluminium seals were used. Extract solution (40 ml) was added to each flask following different temperature at 0, 10, 20, 30, 40, 50, 60 and 70 ± 1°C. The absorbance of the samples was removed for absorbance readings at 480 nm.

### Statistical analysis

Calculations of anthocyanin degradation parameters were obtained and absorbance data were used to build Neperian logarithm graphs from the absorbance/initial absorbance ratio (Equation 1) versus time. This data was subjected to regression analysis and the angular coefficients of straight lines were obtained. Value for degradation velocity (k) per unit time ( $t^{-1}$ ) was obtained through the slope of straight lines. The half-life ( $t_{1/2}$ ) was obtained using the first order Arrhenius equation (Equation 2). The statistical parametric test was used (t for student) to verify the averages of degradation velocity values and the average half-life time.

$$K = \ln[\text{absorbance}(t)/\text{absorbance}(t_0)] \quad (1)$$

$$t_{1/2} = 0.693/K \quad (2)$$

### Measurement of colour and stability

The colour stability of anthocyanin was determined at 25 ± 1°C. The anthocyanin pigments were dissolved in acidified methanol to make stock solutions with concentrations of 1 mg/ml and aliquots of 100 µl of these solutions were transferred to samples tubes, evaporated to dryness and dissolved in 1.5 ml of each of the 14 different buffer solutions ranging from 1.1, 3.0, 4.1, 5.1, 6.0, 6.6, 6.8, 6.9, 7.2, 7.3, 8.0, 8.9, 9.9 and 10.5 to give a final anthocyanin pigment concentration of 1.0 mg/ml.

Each anthocyanin buffer solutions were stored at 25 ± 1°C. The sample tubes were sealed with parafilm and the anthocyanin

pigments were stored under air atmosphere. Absorbance was recorded by ultraviolet/visible (UV/Vis) Spectrophotometer Genesys – 5 at 480 nm. Pure buffers were used as reference cell solutions. UV/Vis measurements (absorbance) were made after 1 h and 1, 2, 3, 4, 5, 6, 13 and 20 days. The colour intensities were measured as absorbance values at 480 nm for anthocyanin and at each pH value and expressed as molar absorptivity ( $\xi$  in  $M^{-1}CM^{-1}$ ).

### Buffer solutions

Buffer solutions of 14 different pH values were prepared and the accurate pH for each buffer solution was measured with a pH meter with pH electrode and a temperature probe. The solvents used for preparations of the buffer solutions were 0.2 M KCl (A), 0.2 M HCl (B), 0.1 M  $KHC_8O_4H_4$  (C), 0.1 M HCl (D), 0.1 M NaOH (E), 0.1 M  $KH_2PO_4$  (F), 0.025 M Borax (G) and 0.05 M  $Na_2HPO_4$  (H). Table 1 shows the solvent proportions.

## RESULTS AND DISCUSSION

### Effect of temperature on red sorghum anthocyanin pigment stability

The effects of light, temperature and pH on the stability of anthocyanins were studied by several authors and relationships between these effects and the decomposition of the anthocyanin pigments has always been observed (Stringheta, 1991; Kuskoski et al., 2000).

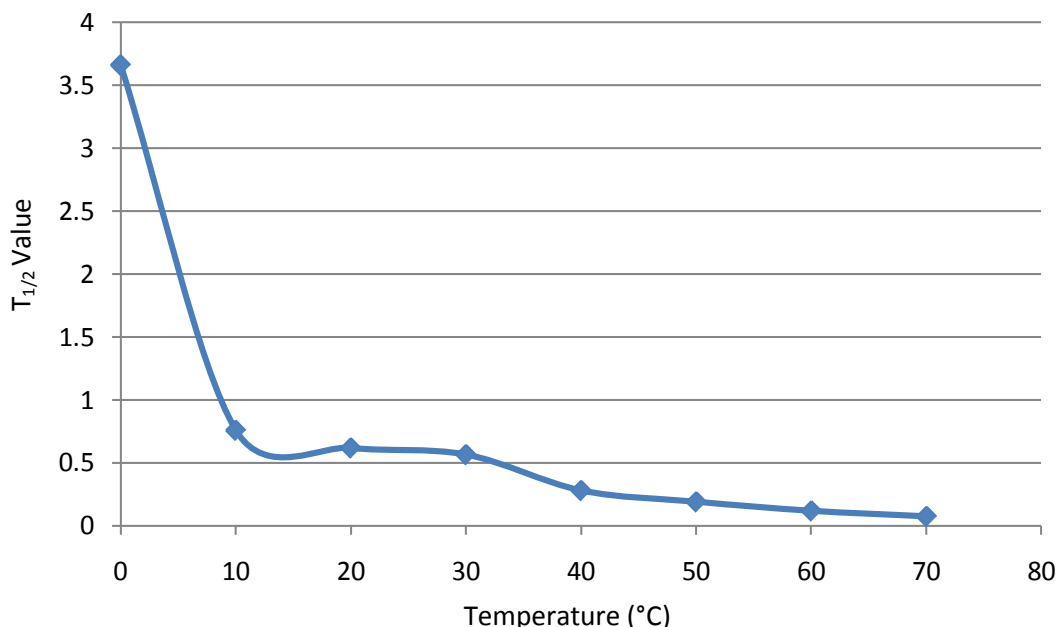
Based on our results, temperature had a great influence on the stability of anthocyanin pigment, both in the presence and absence of light. The sorghum anthocyanin extracted using acidified methanol was more stable than methanol extract. The colour loss in both extracts increased with increase in temperature/time and more so in the presence of light. (Table 1 and Figures 1 to 4) Degradation velocity (K) and half-life ( $t_{1/2}$ ) of sorghum anthocyanins in the presence of light was lower than dark in both the extracts. Mohammed et al. (2006) reported that increasing in time and temperature of heating resulted in changes in anthocyanin and the co-pigmentation complex which resulted an increase in absorbance (Hyperchromic effects) and bathochromic shift in solutions.

Nuzhet et al. (2004) reported that acylated anthocyanins were significantly more stable than non-acylated anthocyanins at all storage temperatures. However, both the extracts were stable when stored at 0°C.

Gous (1989) also reported that a crude black sorghum anthocyanin extract was relatively stable to temperature and light, with no change in absorbance when subjected to 70°C for up to 36 h, and only a 9% reduction in absorbance when subjected to 1000 µ-einstein (equivalent to half-sunlight intensity) at 24°C for 48 h. The high stability, coupled with high levels of the anthocyanins measured in sorghum relative to other commodities, further establishes a need to explore the use of sorghum anthocyanins as food colourants.

**Table 1.** K-value of methanol and acidified methanol extract of red sorghum bran anthocyanin stability at different temperatures (Light and dark).

S/N	Temperature (°C)	K-value			
		Methanol extract		Acidified methanol extract	
		Light	Dark	Light	Dark
1	70	9.32	5.043	1.607	2.451
2	60	5.866	4.474	1.171	2.408
3	50	3.631	2.516	0.902	1.862
4	40	2.470	1.559	0.864	0.794
5	30	1.226	0.632	0.271	0.130
6	20	1.121	0.405	0.196	0.104
7	10	0.913	0.279	0.158	0.092
8	0	0.190	0.108	0.152	0.053

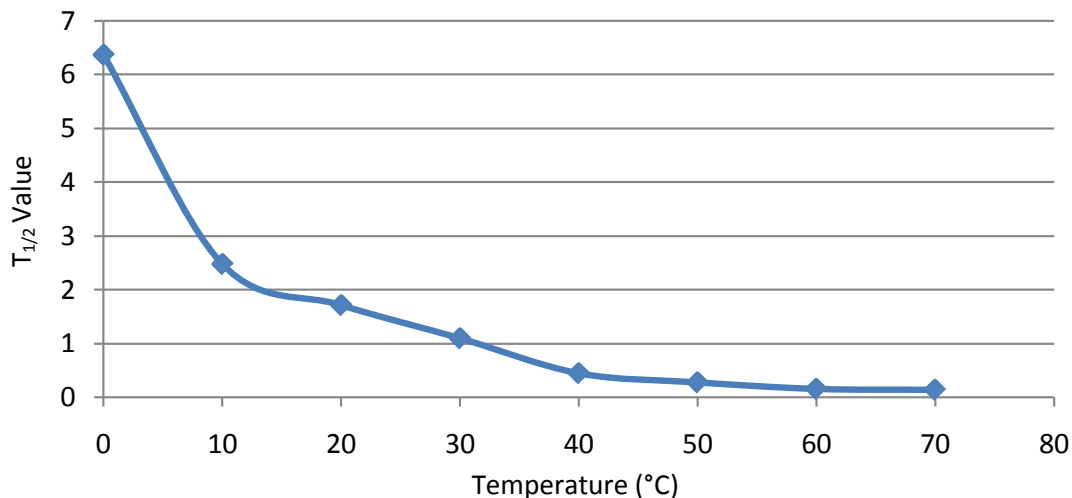
**Figure 1.** The  $t_{1/2}$  value of methanol extract of red sorghum bran anthocyanin at different temperatures (Light).

### Effect of pH on red sorghum anthocyanins

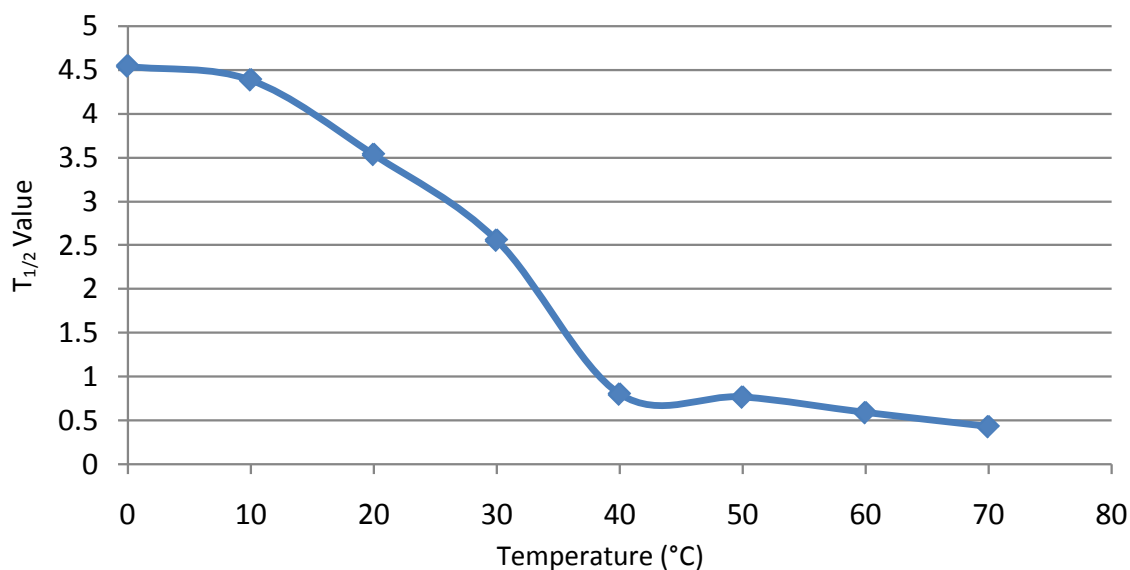
Anthocyanins may exhibit different colours, depending on their structure (for example, glycosylation, acylation), pH and the presence and concentration of co-pigments. At a given pH, equilibrium exists between anthocyanin/aglycone structures: a blue quinoidal (anhydro) base (A), a red flavylium cation ( $AH^+$ ), the colourless carbinol pseudobase (B) and chalcone (C). Under neutral or slightly acidic condition, the anthocyanins exist predominantly in their colourless forms, due to the instability of the anhydro base. The rate of anthocyanin degradation has long been known to be pH dependent.

For example, lowering the pH is largely attributed to the higher concentration of the flavylium cation. Stabilization of the coloured species, especially the quinoidal base (A) could be further conferred through intermolecular (for example, flavonoids, polyphenols) and intramolecular (for example, presence of acyl groups on sugar moieties of the anthocyanin molecule itself) co-pigmentation (Brouillard and Dangles, 1994; Jackman et al., 1987).

Based on our results, pH had a great influence on the stability of anthocyanin pigment. The anthocyanin was more stable at pH 1.1, 8.0, 8.9 and 9.9 in both methanol and acidified methanol extracts of sorghum anthocyanins (Figures 5 and 6). The flavylium cation form



**Figure 2.** The  $t_{1/2}$  value of methanol extract of red sorghum bran anthocyanin at different temperatures (Dark).



**Figure 3.** The  $t_{1/2}$  value of acidified methanol extract of red sorghum bran anthocyanin at different temperatures (Light).

presents anthocyanins that are considered to be stable (Francis, 1992).

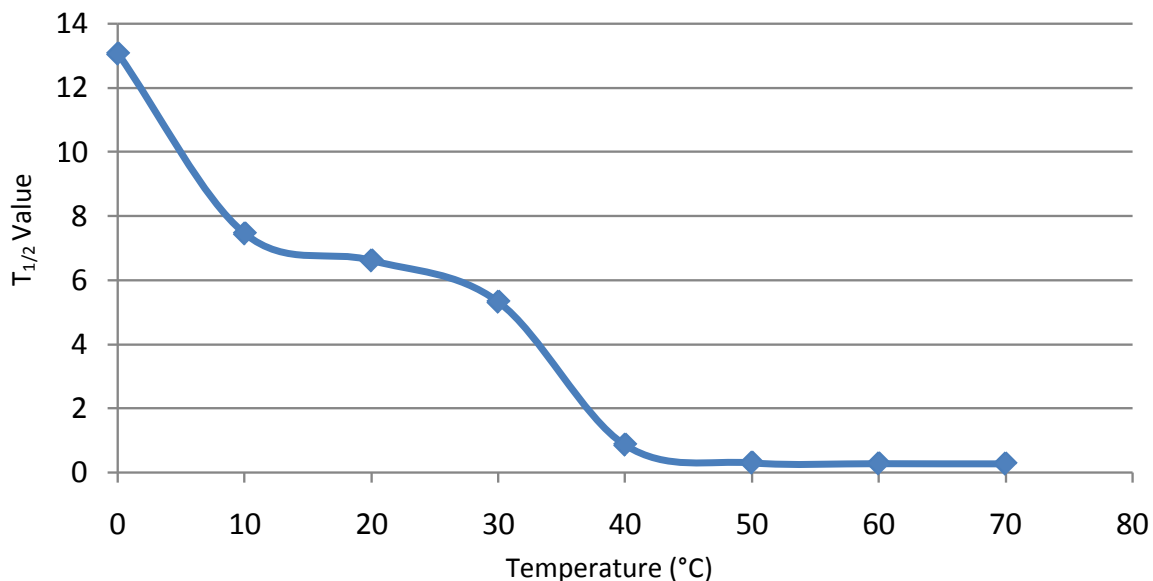
At pH from 3 to 6, there is an important colour loss for anthocyanin alone and significant colour retention for the one containing the anthocyanins and the co-pigment. The colour loss is due to the formation of colourless carbinol pseudobase.

At pH 6.6 to 7.3, the solution contains anthocyanin, where colour increases with increase in absorbance (Figures 5 and 6). At pH 8.0 to 9.9, the colour was retained and the absorbance was increased. In these pH

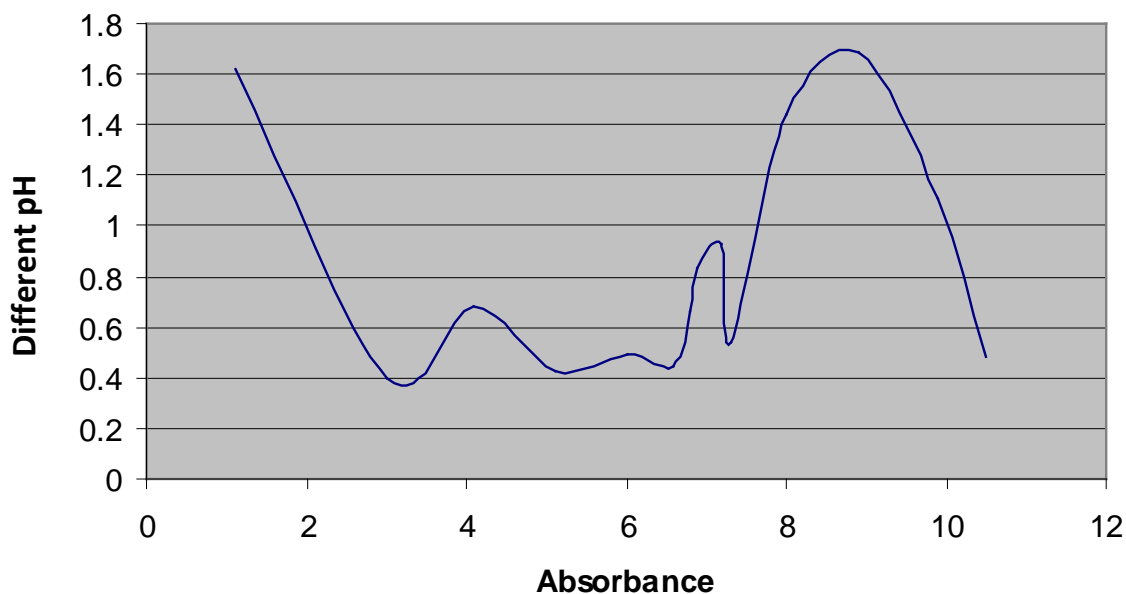
ranges, quinoidal bases were formed and again, colour retention is due to decrease in the amount of the carbinol pseudobase in the solution (Brouillard et al., 1991).

At pH 10.5, the absorbance was decreased followed by decrease in colour (Figures 5 and 6). At this pH, anthocyanins achieved immediately after dissolution bluish or greenish tones, which disappeared very rapidly.

Kjell and Anderson (2005) reported that in acidic solutions, increment in pH produced bathochromic shifts of anthocyanin occurs. Just around neutrality, these shifts were dramatic, with the highest visible  $\lambda$  maximum values



**Figure 4.** Showing the  $t_{1/2}$  value of acidified methanol extract of red sorghum bran anthocyanin at different temperatures (Dark).



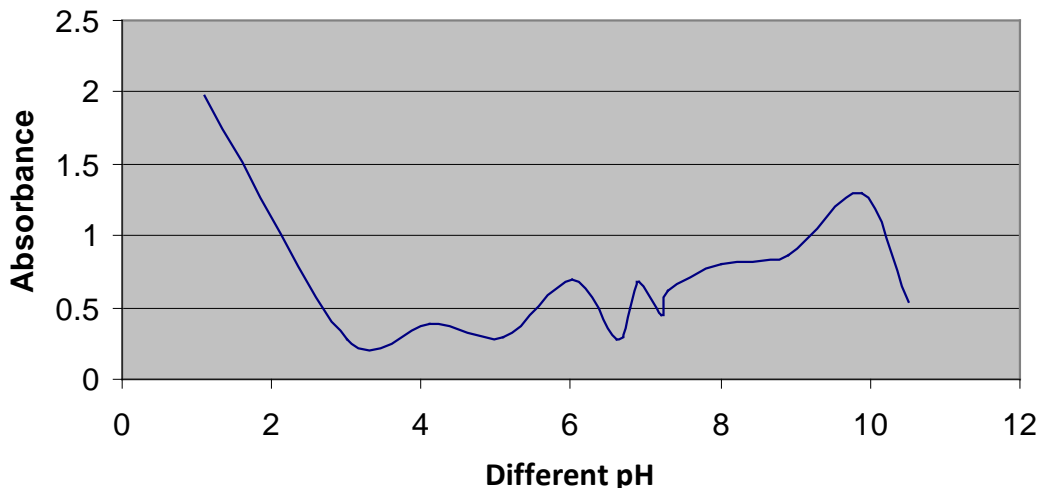
**Figure 5.** UV/Visible spectral absorbance at 480 nm of 0.05 M methanol extract of crude red sorghum bran anthocyanins samples taken initially dissolution in buffered solutions at pH 1 to 14 at temperature of 25°C after 20 days of incubation.

at pH 7.3. Further increase in pH resulted in hypsochromic shifts until new bathochromic shifts were revealed above pH 9.0.

Currently, sorghum is the only known natural food source of 3-deoxyanthocyanin in significant quantities. The 3-deoxyanthocyanins are more stable to light, heat and change in pH than other anthocyanins. Higher pH

stability was observed for crude sorghum 3-deoxyanthocyanins than the deoxyanthocyanidin standards (Awika et al., 2004). Similar results were also observed in our experiment.

However, because most anthocyanins of commercial interest exist naturally in acylated forms, the stability of the acylated anthocyanins related to sorghum



**Figure 6.** UV/Visible spectral absorbance at 480 nm of 0.05 M acidified methanol extract of crude red sorghum bran anthocyanins samples taken initially dissolution in buffered solutions at pH 1 to 14 at temperature 25°C after 20 days of incubation.

3-deoxyanthocyanins. Acylated anthocyanins, mostly with phenolic acids are far more stable than the non-acylated forms (Giusti et al., 1999; Bridle and Timberlake, 1997; Eiro and Heinonen, 2002) and are the major natural forms of anthocyanins that are currently used commercially as food pigments, even though the 3-deoxy anthocyanins exist in nature as aglycones were reported in sorghum. The sorghum 3-deoxy anthocyanins extracts are apparently more pH stable than even the acylated forms of anthocyanins from other sources.

Various authors have reported on the colour stability of 3-deoxyanthocyanidins relative to anthocyanidins (Sweeny and Iacobucci, 1983; 1981; Mazza and Brouillard, 1987).

This was the first report on the effect of temperature and pH on stability of anthocyanin from red sorghum bran. The current drive toward use of anthocyanins is largely motivated by the fact that they are "natural" forms of 3-deoxyanthocyanidins and are more stable. We hope that our results will stimulate a strong interest in these unique anthocyanidins as food colourants and for other biological applications. Stability of a coloured compound is very important in retaining the appearance of the product in which it is incorporated and hence the customer acceptance, as well as the bioefficacy.

## Conclusion

Pigmented sorghums are a rich source of unique 3-deoxyanthocyanidins that give yellow to orange colour in acidic media. The red sorghum bran anthocyanins were more stable to temperature, light and pH-induced colour loss than the anthocyanins commonly found in fruits and vegetables. These sorghum 3-deoxyanthocyanins are a

potentially valuable source of natural food colour. Additional studies are necessary to establish the colour stability of the anthocyanins in actual food systems. We are currently carrying out detailed analyses to identify all the anthocyanins and their biological properties of red sorghum bran anthocyanins.

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