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ABSTRACT

Extraction of antioxidant biocolorant pigments from leaf sheaths of dye sorghum was optimized. Effects of temperature and ethanol concentration of the extraction solvent on the concentrations of the 3-deoxyanthocyanidins, total phenolics and total anthocyanins, and the colour parameters of the bio-colorant extract were evaluated using the response surface methodology. Extraction parameters affected the extraction rate of the biocolorant pigments and the colour characteristics of the extract. Maximum pigment yields were obtained at 50 °C and an ethanol concentration of the solvent of 51 mL 100 mL^{-1}. Addition of HCl (1 mL 100 mL^{-1}) to the solvent significantly improved the extractability of the bio-colorant pigments. The crude extract from the leaf sheaths showed high antioxidant capacity with a total antioxidant capacity of 1026 mg of Trolox equivalent (TE) g^{-1} of DM.

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1. Introduction

The demand for natural dyes is increasing steadily because of environmental pollution and toxicity associated with the use of synthetic dyes (Magda, 2003). Natural colourants have attractive bright colours and a history of safe use (Bao, Cai, Sun, Wang, & Corke, 2005). In addition, certain functional properties, i.e. their antioxidant activity and associated health benefits, including anti-cancer and chemoprotective properties (Karaivanova, Drenska, & Ovcharov, 1990), make them of particular interest to processors and consumers. The food industry is therefore challenged to replace synthetic dyes by natural ones (Bao et al., 2005). Presently, considerable research is directed towards the identification and characterisation of pigments from plant sources such as vegetables (Giusti, Wrolstad, & Gloria, 2001), fruits and flowers (Awika, Rooney, & Waniska, 2004). Sorghum spp. are cereals, which are important food sources in Africa (Dicko, Gruppen, Traore, Voragen, & Van Berkel, 2006). However, not all sorghum is destined for food; in Benin, some farmers' varieties, referred to as dye sorghums, are mainly grown for the pigments in the leaf sheaths adjacent to the stem of the plant (Boon, Engels, Struik, & Cone, 2005). The production of dye sorghum is primarily the domain of rural women, who derive a direct benefit from the commercialization of the leaf

sheaths. Dye sorghum has been used in Benin and other African countries for centuries (Kayodé et al., 2011). Traditionally, the watery dye extract from the leaf sheaths is used as bio-colouring for foods (e.g., local cheese, porridge), lick stones for cattle, leather, wickerwork, ornamental calebashes, and in traditional medicine. The derived food products have an attractive red colour, which is highly appreciated by consumers. In addition, local people use dye sorghum to prepare a red infusion to treat anaemia and menstrual disorders. Recently, Kayodé et al. (2011) showed that apigeninidin and luteolinidin (both 3-deoxyanthocyanidins) are the predominant anthocyanins in the leaf sheaths of dye sorghum. The greater stability of 3-deoxyanthocyanins compared to anthocyanins is a major reason for the interest in their use as natural colourants (Awika & Rooney, 2004). The 3-deoxyanthocyanidins offer many applications in food, beverages and pharmaceutical industries (Awika & Rooney, 2004). They are recognized as health-promoting phytochemicals, e.g., it has been demonstrated that they are more cytotoxic to human cancer cells than the 3-hydroxylated antho-cyanidin analogues (Shih et al., 2007). Recently Yang, Browning, and Awika (2009) demonstrated that the sorghum 3-deoxyanthocyanins possess strong Phase II enzyme inducer activity and cancer cell growth inhibition properties. In the West African region, the biocolorants from the leaf sheaths of dye sorghum are currently extracted using boiling water or cold water containing kanwu, a mineral complex. These methods are not effi-cient and do not result in an optimum extraction and quality of the

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pigments. In industry, a solvent-extraction procedure is commonly used to extract phenolic compounds and the concentration of solvent, time, and temperature are important process parameters (Spigno, Tramelli, & De-Faveri, 2007). As to different organic solvents, ethanol—water and acetone—water mixtures resulted in similar yields for total phenols, which were higher than obtained with ethylacetate—water mixtures (Vatai, Škerget, & Knez, 2009). Ethanol is a more environment-friendly solvent, which additionally benefits from the GRAS (Generally recognized as safe) status.

The present study aimed at improving the extraction and quality of biocolorant phenolics from leaf sheaths of dye sorghum. More specifically, the objective was to evaluate the effect of two process variables, i.e. temperature and ethanol concentration of the extraction solvent, on the extraction yield of 3-deoxyanthocyanidins, total anthocyanins and total phenolics, and the colour characteristics and antioxidant activity of the biocolorant extract. As it is likely that the effect of one process variable depends on the other, interactions between factors need to be taken into account. As interactions will not be detected using a one-factor-at-a-time approach (Giovanni, 1983), we used a research design methodology that is able to detect such interactions, i.e. response surface methodology (RSM) with a central composite design. Central composite designs are the basis for RSM and are used to estimate parameters of a full second-degree model. Such a quadratic model is usually sufficiently accurate for product and process design (Giovanni, 1983).

2. Materials and methods

2.1. Plant material

Leaf sheaths of a farmers' variety of dye sorghum, locally known as *Abokoun vitamin*, were collected from fields in *Dassa-Zounmè* (Lat. °N: 7°55′, Long. °E: 1°58′), Republic of Bénin. The crop was grown in 2009 under natural conditions characteristic of the Guinea Savannah climate of West Africa. After harvesting, the leaf sheaths were dried at 50 °C for 6 h to a moisture content of 7—9 g 100 g^{-1} (w/w), and they were ground to powder using an Ultra Centrifugal Mill (Retsch GmbH, Haan, Germany) with a 1 mm sieve and subsequently stored at −20 °C until use.

2.2. Experimental design

Response Surface Methodology is a statistical method that uses quantitative data derived from an appropriate experimental design with quantitative factors to estimate the relationship between a response and the factors in order to optimize processes or products (Giovanni, 1983). In this study an orthogonal rotatable central composite design (Montogomery, 2001) for $K = 2$ factors was used to estimate the simultaneous effect of two process variables on the apigeninidin, luteolinidin, total anthocyanins and total phenolics concentrations, and the colour characteristics of the biocolorant extract in a quadratic function. The variables (factors) were the temperature (25—75 °C), and ethanol concentration of the solvent (0—100 mL 100 mL^{-1}). The responses were apigeninidin, luteolinidin, total anthocyanins, total phenolics concentrations and colour parameters (L, a*, b* and ΔE). The design generated 14 observations, which were distributed as follows: 4 kernel points, 4 star points and 6 replications at the central point. The design matrix and variable combinations are presented in Table 1.

2.3. Extraction

Samples were extracted in ethanol/H$_2$O with various concentrations as predefined in the experimental design (Table 1). Thirty mg of powder was extracted with 10 mL of solvent at temperatures

Table 1
Matrix of the model and combination of variables.

Treatment code	Level codes		Level of the variable	
	Temperature (°C)	Solvent	Temperature (°C)	Solvent[a]
1	0	0	50	50
2	0	0	50	50
3	0	0	50	50
4	0	0	50	50
5	0	0	50	50
6	0	0	50	50
7	+1	+1	65.0	80.05
8	+1	−1	65.0	19.95
9	−1	−1	35.0	19.95
10	−1	+1	35.0	80.05
11	0	+α	50	100
12	+α	0	75	50
13	0	−α	50	0
14	−α	0	25	50

[a] mL 100 mL^{-1} ethanol in H$_2$O.

as defined in the experimental design, under agitation for 30 min using flat-bottom glass tubes equipped with a magnetic stirrer. The mixtures were centrifuged at 2500 g for 10 min and the supernatants were collected. The residues were re-extracted twice under the same conditions, resulting in 30 mL crude extract. All extracts were used as they were after centrifugation for various analyses. Commonly, phenolic pigments are extracted at laboratory level using an acidified solvent. Thus, we added HCl (1 mL 100 mL^{-1}) to the optimum extraction solvent mixture as generated by the model.

2.4. Physico-chemical analysis

2.4.1. Total anthocyanins (ACY) determination

Total anthocyanins concentration was calculated as described by Abdel-Aal and Hucl (1999) using cyanidin 3-glucoside as a standard pigment. The absorbance of the pooled extracts was measured after centrifugation at 525 nm against a reagent blank. ACY was expressed as mg cyanidin 3-glucoside equivalent per 1 g powder based on DM.

2.4.2. Total phenolics (TPC) determination

TPC were measured following the method of Singleton and Rossi (1965), modified as follows: to 300 μL of extract, 4.2 mL of distilled water, 0.75 mL of Folin-Ciocalteu's reagent (Merck, Germany) and 0.75 mL of sodium carbonate solution (200 g L^{-1}) were added. After incubation for 30 min, the optical density was measured at 760 nm against a reagent blank. Gallic acid was used as standard and the results were expressed as gallic acid equivalent (GAE) g^{-1} of sample DM.

2.4.3. Determination of 3-deoxyanthocyanidins

The absorbance of the pooled extracts was measured (6715UV/Vis. Spectrophotometer JENWAY) after centrifugation at 468 nm (for apigeninidin) and 482 nm (for luteolinidin) against a reagent blank. The optical density was expressed g^{-1} of powder DM.

2.4.4. Colour measurements

Colour of pooled extracts was measured with a Minolta CR-210 portable chromameter (Illuminant D65 CIE 1976) in reflective mode, standardized with a standard white tile ($Y = 94.8$, $x = 0.315$ and $y = 0.3324$). L*, a*, b* and ΔE values were recorded.

2.4.5. Determination of antioxidant capacity

Extracts were analyzed for their total antioxidant capacity by the ABTS radical cation scavenging assay (Trolox equivalent antioxidant capacity (TEAC)) and the ferric reducing antioxidant power (FRAP) assay.

The ABTS assay was carried out following the method modified by Pellegrini, Del Rio, Colombi, Bianchi, and Brighenti (2003) and Moore et al. (2005). A stable stock solution of ABTS radical cation was produced by reacting a 3.84 g L^{-1} aqueous solution of ABTS with 0.66 g L^{-1} potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12—16 h before use. On the day of analysis, an ABTS radical cation working solution was obtained by diluting the stock solution in ethanol to an absorbance of 0.70 ± 0.02 Absorbance Unity (AU) at 734 nm. Hundred microlitres of extract were mixed with 1.25 mL of the ABTS working solution and absorbance was read at 734 nm after a 1 min reaction time. Results were expressed as TEAC in g Trolox g^{-1} leaf dry matter (DM).

The FRAP assay is based on the reduction of the Fe^{3+}—TPTZ (2,4,6-tripyridyl-s-triazine) complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 595 nm (Benzie & Strain, 1999). Briefly, 0.2 mL of sample extract was mixed with 1.3 mL of the FRAP reagent. Absorption was measured at 595 nm in a spectrophotometer (6715UV/Vis. JENWAY) after 30 min of incubation at 37 °C. The FRAP reagent was prepared fresh daily and consisted of acetate buffer (3.1 g sodium acetate trihydrate in 16 mL glacial acetic acid, made up to 1 L with distilled water, pH 3.6), TPTZ (0.0312 g TPTZ in 10 mL HCl), and $FeCl_3$ (0.5410 g $FeCl_3 \times 6H_2O$ in 100 mL distilled water) in a ratio of 10:1:1 (v/v/v). FRAP values were obtained by comparing the absorption change in the test mixture with doses obtained from increasing concentrations of Fe^{3+} and expressed as g of Fe^{2+} equivalents g^{-1} leaf sheaths DM.

2.5. Statistical analysis

Data were analyzed using the Minitab 14 statistical program. A second order polynomial model was used to describe the relationship between the responses (Y) and the variables (X) as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_5x_1x_2$$

in which b_0 is a constant, b_1 and b_2 are linear effect coefficients, b_3 and b_4 are quadratic effect coefficients, and b_5 is an interaction effect coefficient. The fitted polynomial equations were expressed in a 3D response surface in which the response is presented on the vertical axis and two factors on the two horizontal perpendicular axes.

3. Results and discussion

3.1. Effect of extraction parameters on the concentration of 3-deoxyanthocyanidins

The responses of the model for apigeninidin, luteolinidin, total anthocyanins, total phenolics concentrations and colour parameters

(L, a*, b* and ΔE) are presented in Table 2. The polynomial equation fitting and the estimated linear regression coefficients are presented in Table 3. The apigeninidin concentration in the extracts ranged from 3.28 to 8.81 AU g^{-1} of leaf sheath DM. The concentration of luteolinidin varied from 3.41 to 9.12 AU g^{-1} (Table 2). Analysis of variance revealed significant ($p \leq 0.05$) effects of extraction temperature (X_1) and solvent (X_2) on the yield of the 3-deoxyanthocyanidins. Particularly, the linear and the quadratic as well as the interactive effects of these factors were significant with respect to the 3-deoxyanthocyanidins concentration of the extracts (Table 3). Fig. 1a shows the trends in apigeninidin concentration of the extracts as function of temperature, the solvent composition, and their interaction. The apigeninidin concentration of the extracts increased with temperature and ethanol concentration in the solvent, especially in the temperature range between 25 and 50 °C and ethanol concentrations from 0 to 50 mL 100 mL^{-1}. Similar trends were observed for luteolinidin concentration in the extracts (Fig. 1b).

3.2. Effect of extraction parameters on the total anthocyanins concentration

The highest value for total anthocyanins (7.35 AU g^{-1}) was obtained from treatment 12 (i.e. 75 °C and an ethanol concentration of 50 mL 100 mL^{-1} solvent), and the lowest (3.08 AU g^{-1}) from treatment 13 (50 °C and no ethanol in the solvent) (Table 2). Both the temperature and the ethanol concentration of the solvent affected the extraction of the total anthocyanins (Table 3). The linear and quadratic effects of these factors, as well as their interactive effects ($p < 0.01$), affected the anthocyanins concentration of the extract. Malien-Aubert and Amiot-Carlin (2006) reported that the temperature and the type of solvent influence the extraction rate of anthocyanin pigments. Likewise, Spigno et al., (2007) indicated that the recovery of phenolic compounds is influenced by the concentration of solvent, extraction time, and temperature. Fig. 1c shows the changes in the anthocyanins concentration of the extract as a function of the temperature, solvent and their interaction. Trends for the anthocyanins were similar to those for the 3-deoxyanthocyanidins; the anthocyanins concentration tends to increase with higher temperatures and ethanol concentrations in the solvent. This effect is significant at temperatures ranging from 25 to 50 °C and ethanol concentrations from 0 to 50 mL 100 mL^{-1}.

3.3. Effect of extraction parameters on the total phenolics concentration

The phenolics concentration of the extracts varied among treatments and the major variation was explained by the model.

Table 2
Model response for apigeninidin, luteoninidin, total anthocyanins, total phenolics and colour characteristics of the extracts.

Treatment code	Absorbance (AU g^{-1})			Total phenolics (g 100 g^{-1})	Colour			
	Apigeninidin	Luteolinidin	Anthocyanins		L*	a*	b*	ΔE
1	7.48	7.67	6.45	32.1	27.6	3.8	−0.2	69.6
2	7.12	7.39	6.08	33.4	27.8	3.7	−0.4	69.5
3	7.02	7.22	5.93	31.2	27.9	4.2	0.2	69.5
4	7.53	7.77	6.5	33.3	27.8	4.0	−0.1	69.5
5	7.71	7.84	6.31	35.4	27.8	4.1	0.3	69.6
6	6.56	6.99	5.66	35.6	27.9	4.2	0.4	69.4
7	8.81	8.54	7.32	27.3	28.1	5.6	−0.4	69.4
8	7.27	7.34	6.53	34.0	26.7	4.0	−0.3	70.7
9	3.36	3.62	2.83	19.7	32.2	3.8	2.9	65.5
10	8.27	9.12	6.73	41.1	29.3	5.4	0.1	68.3
11	6.89	7.57	6.33	22.1	28.4	5.5	−0.2	69.0
12	8.28	8.21	7.35	37.6	28.1	4.9	−0.5	69.3
13	3.28	3.41	3.08	10.3	29.1	3.3	1.4	68.4
14	7.41	8.0	5.64	29.2	29.6	3.5	1.3	67.7

Table 3
Values of the coefficients in the model and their significance.

Coefficients	Apigeninidin	Luteolinidin	Anthocyanins	Total phenolics	L*	a*	b*
b_0	−0.9262	−1.2516	−2.3134	−28.0567	43.5865	3.9321	9.4854
b_1	0.0236*	0.0343*	0.0762**	0.7157	−0.3814**	−0.0393*	−0.1913***
b_2	0.2195***	0.2439***	0.1780***	1.5423***	−0.1739	0.0013***	−0.1196**
b_3	0.0011	0.0011	0.0006	0.0016	0.0020	0.0006	0.0007
b_4	−0.0008**	−0.0008**	−0.0006**	−0.0065***	0.0005	0.0002	0.0003
b_5	−0.0019*	−0.0024**	−0.0017**	−0.0155***	0.0024*	−0.0000	0.0015**
R^2	0.84	0.9	0.91	0.89	0.68	0.76	0.85

b_0, constant; b_1 and b_3 coefficients for temperature; b_2 and b_4 coefficients for solvent; and b_5, coefficient for interaction (temperature × solvent). *Significant at $p < 0.05$. **Significant at $p < 0.01$. ***Significant at $p < 0.001$. Data reported in this table are the measured (fitted) values of the coefficients b_0, b_1, b_2, b_3, b_4 and b_5, which are explained in detail in the statistical analysis section.

The coefficient of variation (R^2) was 0.89 for total phenolics concentration in the extracts. The highest concentration of total phenolics (41.1 g 100 g^{-1}) was obtained from treatment 10 (i.e. 35 °C, and an ethanol concentration of 80 mL 100 mL^{-1} solvent) and the lowest (10.4 g 100 g^{-1}) from treatment 13 (i.e. 50 °C and no

ethanol in the solvent). The analysis of variance showed that both temperature and solvent exerted a significant effect on the phenolics concentration of the extracts. The linear and quadratic terms of these parameters significantly ($p < 0.001$) contributed to the outcome of the model (Table 3). Moreover, the interaction

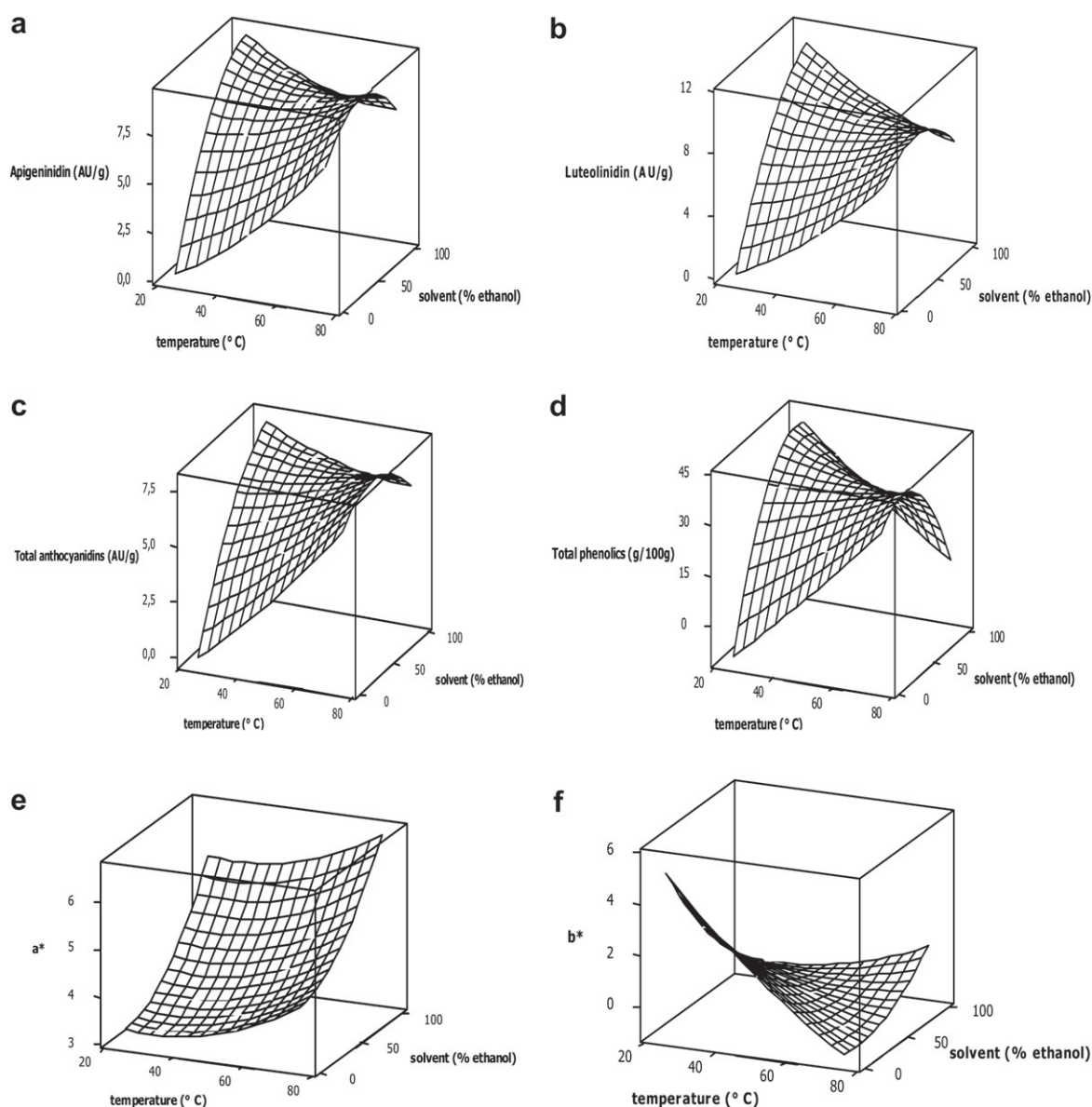**Fig. 1.** Response surface curves showing the effects of ethanol concentration of the solvent (mL 100 mL^{-1}ethanol in H$_2$O) and temperature on (a) apigeninidin, (b) luteoninidin, (c) total anthocyanins, (d) total phenolics, (e) a* and (f) b* of biocolorant extract.

Table 4
Pearson correlation matrix of variables.

	Total anthocyanins	Apigeninidin	Luteolinidin	Total phenolics	a*
Apigeninidin	0.977**				
Luteolinidin	0.951**	0.984**			
Total phenolics	0.756**	0.788**	0.804**		
a*	0.594*	0.553*	0.564*	0.280	
b*	−0.897**	−0.809**	−0.771**	−0.587*	−0.476

*significant at 0.05, **significant at 0.001.

between temperature and solvent was also found to be significant. Fig. 1d shows the trend in extracted total phenolics as a function of temperature, solvent composition, and their interaction. At temperatures between 25 and 50 °C and solvent ethanol concentrations of 0–50 mL 100 mL^{-1}, the total phenolics concentration of the extract increased significantly. Temperatures above 50 °C resulted in lower phenolics concentration. The trend in phenolics concentration is comparable to that of the anthocyanins, which are also phenolic substances (Macheix, Fleuriet, & Billot, 1990). Clearly, the phenolic pigments are poorly extracted from the leaf sheaths using an aqueous solvent with a low alcohol concentration. However, the watery extraction can be improved by heating the mixture (interactive effect).

3.4. Effect of extraction parameters on colour characteristics of the extracts

Colour is an important quality criterion with respect to the commercial value of colourant products. Anthocyanins are responsible for the red colour of many plant species Cheynier and Sarni-Manchado (2006). Commonly, colorimetric parameters used to characterize colourants are Chroma (C*) and hue angle (h*) (Malien-Aubert & Amiot-Carlin, 2006). These parameters are derived from the CIELAB a* and b* coordinates which were used in the present study. Analysis of variance (Table 3) revealed that both temperature and solvent affect the colour characteristics of the extracts. In general the coordinate a* of the extract increases with extraction temperature and ethanol concentration (Fig. 1e and f). The highest value of a* (5.6) was obtained for treatment 7 (65 °C and an ethanol concentration of 80 mL 100 mL^{-1} solvent). In contrast, we recorded the highest values of b* for lower temperatures and lower amounts of ethanol in the solvent.

3.5. Relationship between anthocyanin, phenolic compounds and colour characteristics

The Pearson correlation matrix (Table 4) shows that the yield of total phenolics is positively correlated with that of total anthocyanins ($r = 0.756$; $p < 0.01$), apigeninidin ($r = 0.79$, $p < 0.01$) and luteolinidin ($r = 0.80$; $p < 0.01$). This highly significant correlation can be explained by the fact that anthocyanins belong to the
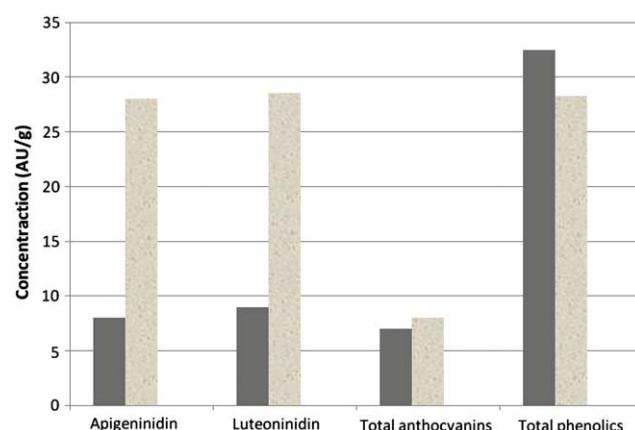

Fig. 2. Effect of HCl on the extractability of dye sorghum phenolic compounds: ■: extraction solvent (Ethanol/H$_2$O: 50 mL 100 mL^{-1}) □: extraction solvent (Ethanol/H$_2$O: 50 mL 100 mL^{-1} + 1 mL 100 mL^{-1} HCl).

phenolic compounds (Macheix, Fleuriet, & Billot, 1990). Total anthocyanins significantly correlated with amounts of apigeninidin ($r = 0.98$, $p \leq 0.01$) and luteolinidin ($r = 0.95$, $p \leq 0.01$) as previously reported (Kayodé et al., 2011). This could also be expected, since both apigeninidin and luteolinidin belong to the anthocyanins with the distinctive feature that they lack an oxygen molecule at the C-3 position (Awika et al., 2004). Total anthocyanins positively correlated with a* ($r = 0.594$; $p < 0.05$) and negatively with b* ($r = -0.897$; $p < 0.01$).

3.6. Optimization of the extraction conditions

To determine the extraction conditions that result in maximum yields of anthocyanin pigments, we optimized the amount of ethanol in the solvent and temperature of extraction. The target characteristics of the extract were as follows: apigeninidin 6.5–7.3 AU g^{-1}; luteolinidin 7.0–7.5 AU g^{-1}; total anthocyanins 5.5–6.2 AU g^{-1}; total phenolics 29–34 g 100 g^{-1}; a* 3.5–4.1; and b* −0.2 to 0.1. According to our model, the extraction conditions for the targeted values should be an extraction temperature of 50 °C in combination with an ethanol concentration of solvent of 50.8 mL 100 mL^{-1} with a desirability of 0.96. To check the predictive value of the mathematical model, we conducted additional experiments at the calculated optimum extraction conditions. The predicted and the experimental outcomes for anthocyanins, apigeninidin, luteolinidin, total phenolics and colour characteristics are presented in Table 5. The experimental and the predicted values are in close agreement with a desirability ranging between 0.81 and 1.0. A Chi-square test indicated that the observed values did not differ significantly from the predicted values. Consequently, the generated model adequately predicts concentration of anthocyanin pigments in the extract. The extraction of the 3-deoxyanthocyanins

Table 5
Predicted and experimental values.

Variables	R^2	Adjusted R^2	F value	P value	Optimal extraction conditions		Optimal values	
					Temperature (°C)	Solvent*	Predicted	Experimental
Apigeninidin (AU g^{-1})	0.90	0.84	15.01	0.001	50	50.79	7.3	7.2
Luteolinidin (AU g^{-1})	0.94	0.90	23.46	0.000	50	50.79	7.54	7.43
Total anthocyanins (AU g^{-1})	0.94	0.91	26.13	0.000	50	50.79	6.19	6.12
Total phenolics (g 100 g^{-1})	0.93	0.89	22.18	0.000	50	50.79	33.99	32.99
a*	0.85	0.76	9.24	0.004	50	50.79	4.1	4.0
b*	0.91	0.85	15.66	0.001	50	50.79	0	0.1

*mL 100 mL^{-1} ethanol in H$_2$O.

Table 6
Comparison of dye sorghum to other plant commodities for their antioxidants properties.

Commodity	Total antioxidant capacity		Source
	FRAP (mg g^{-1} DM)	TEAC (mg g^{-1} DM)	
Leaf sheaths of dye sorghum	49.7	1026.2	This work
Black sorghum bran	–	65.3–100.1	Awika et al. (2004)
Black rice	2.0–4.2	12.5–30.1	Sompong, Siebenhandl-Ehn, Linsberger-Martin, and Berghofer (2011)
Red rice	0.5–4.5	5.2–30.8	Sompong et al. (2011)
Blueberry commercial wild type	6.5	11.4	Dragović-Uzelac et al. (2009), Garzón, Narváez, Riedl, and Schwartz (2010), Denev et al. (2010)
Red raspberry	1.1	5.4	Çekiç and Özgen (2010)
Strawberry	–	3.5	Tulipani et al. (2008)
Red cabbage	–	2.5–3.1	Podsędek, Sosnowska, Redzynia, and Koziolkiewicz (2008); Scalzo, Genna, Branca, Chedin, and Chassaigne (2008)
Red onion	–	14.6	Gorinstein et al. (2009)

significantly improved when HCl (1 mL 100 mL^{-1}) was added to the extraction solvent (Fig. 2). Vatai et al. (2009) reported that acidified extraction media resulted in higher anthocyanin concentrations in the extracts from elder berry and different grape marc varieties. The plant material used in this study (leaf sheaths) is rich in cellulose and lignin. Thus, the incorporation of acid in the extraction solvent might have improved disruption of the plant tissue and thus allowed a better release of pigments.

3.7. Antioxidant properties

Plant pigments, as well as other phytochemicals, have been associated with beneficial health effects, such as prevention of cardiovascular diseases and cancer (Karaivanova et al., 1990). In this context, extracts of leaf sheaths were assessed for their antioxidant properties. The concept of the total antioxidant capacity represents the ability of different antioxidants in scavenging free radicals. The extracts showed very high antioxidant capacity compared to other dye sources (Table 6). High ABTS radical scavenging ability (0.93–1.2 g of Trolox equivalent g^{-1}) was found in the leaf sheaths. FRAP values (0.04–0.06 g g^{-1}) were lower than the respective TEAC values, but remain very high when compared to other dye sources like fruits and vegetables for which TEAC values ranged between 0.0025 and 0.0147 g g^{-1} and FRAP values between 0.0011 and 0.0065 g g^{-1} (Table 6). Clearly, the dye sorghum largely surpasses the other plant commodities for its antioxidant capacity. The dye sorghum showed a comparative advantage as anthocyanin and antioxidant source when compared to cereal bran and fruits and vegetables.

4. Conclusion

Both temperature and ethanol concentration of the extraction solvent significantly affected the yield of biocolorant pigments in the extract of leaf sheaths of dye sorghum. The linear, the quadratic and the interaction effects of these parameters on the extractability of pigments have been elucidated. The highest extraction yield of anthocyanin pigments from leaf sheaths was achieved at an extraction temperature of 50 °C and 51 mL 100 mL^{-1} of ethanol concentration of the solvent. The anthocyanins yield increased by a factor 2 when compared to the traditional watery extraction method. The extraction of the 3-deoxyanthocyanidins can be significantly improved if HCl (1 mL 100 mL^{-1}) is added to the extraction solvent. The crude extract from the leaf sheath also showed very high antioxidant activity.

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