LWT - Food Science and Technology 90 (2018) 592–597

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effects of processing and storage on the stability of the red biocolorant apigeninidin from sorghum

F.U.G. Akogou[a,b,c], A.P.P. Kayodé[a], H.M.W. den Besten[c], A.R. Linnemann[b,*], V. Fogliano[b]

[a] Laboratory of Valorization and Quality Management of Food Bio-Ingredients (LaBio), DNSA/FSA, Université d'Abomey-Calavi, 01 BP 526 Cotonou, Benin
[b] Food Quality and Design, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[c] Laboratory of Food Microbiology, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands

ARTICLE INFO

Keywords:
Apigeninidin
Heat treatment
pH
Colour density
Biocolorant

ABSTRACT

A major drawback to the industrial application of many biocolorants is their instability to processing conditions, thereby limiting their use to replace artificial colorants. 3-Deoxyanthocyanidins have promising features to ensure colour stability in food processing conditions. This study evaluated the stability of apigeninidin, the main 3-deoxyanthocyanidin from sorghum leaf sheaths, to food processing conditions in watery extracts and in a maize porridge. Apigeninidin was not soluble at pH 5.04 ± 0.02. However, apigeninidin was soluble and stable at pH 6–10 with increased colour density and resistance to bleaching at alkaline pH. A heat treatment of 121 °C/30 min degraded 61% of the anthocyanins. At 65 °C, degradation rate of apigeninidin was four times lower at pH 9.03 ± 0.04 than 6.08 ± 0.02. Storage at room temperature promoted endothermic degradation reactions. Nevertheless, photodegradation of apigeninidin was not observed during storage. In the maize porridge, thermal stability of apigeninidin and redness were similar at pH 4–6 whereas they were higher at pH 9.03 ± 0.04. In summary, the watery extract of apigeninidin from sorghum leaf sheaths showed good stability regarding common industrial processes. Nevertheless, the biocolorant's precipitation at pH 5.04 ± 0.02 and degradation at pH 6.08 ± 0.02 and 9.03 ± 0.04 need further investigation to optimise its industrial applications.

1. Introduction

Anthocyanins (ACY) are plant pigments with colours that range from scarlet to blue (Wallace & Giusti, 2015). In solution, ACY are a mixture of the coloured (i.e. the flavylium cation and quinoidal base) and colourless forms (i.e. the carbinol pseudobase and the chalcone) (Brouillard, 1982). The proportion of flavylium cation, quinoidal base, carbinol pseudobase and chalcone at equilibrium in an ACY solution are a function of ACY structures and processing conditions (*viz.* pH, light exposure and temperature) (Mazza & Brouillard, 1987). In general, an increasing pH leads to (a) an increasing hydration of the flavylium cation into carbinol pseudobase and (b) increasing tautomerisation of carbinol pseudobase into chalcone (Brouillard, 1982). Consequently, the proportion of chalcone would be higher than that of the flavylium cation for pH values above 3 (Brouillard, 1982). However, the pH limit for a dominant proportion of chalcone is higher for ACY with a methoxylation or a glycosylation (Brouillard, 1982). In addition, a high temperature during processing or storage increases the rate of endothermic reactions (e.g. hydration of the flavylium cation and tautomerisation of the carbinol pseudobase) (Brouillard, 1982). Furthermore, light exposition of ACY leads to photodegradation of flavylium cations into colourless forms (i.e. the carbinol pseudobase and chalcone) (Dyrby, Westergaard, & Stapelfeldt, 2001).

The 3-deoxyanthocyanidins are a particular class of ACY because the deprotonation constant of its flavylium cation is higher than for the hydration. Consequently, a solution of 3-deoxyanthocyanidins stays coloured at high pH. The 3-deoxyanthocyanidins have the interest of the food industry for their resistance to (a) pH changes (Ojwang & Awika, 2008), (b) bleaching additives (e.g. sulphites) (Ojwang & Awika, 2010), and (c) ring fission during heat treatment (Yang, Dykes, & Awika, 2014). Moreover, they show a better colouring efficiency than the majority of the anthocyanins (Awika, Rooney, & Waniska, 2004) and their colour stability improves in the presence of phenolic acids (Awika, 2008). Most experiments on the stability of 3-deoxyanthocyanidins were with non-food grade solvents such as ethanol, methanol, hydroalcoholic solutions, water acidified with HCl or 70% aqueous acetone (Awika, 2008; Awika et al., 2004; Kayodé, Bara, Dalodé-Vieira, Linnemann, & Nout, 2012; Ojwang & Awika, 2008). Limited data exist on the stability of apigeninidin in watery extracts as commonly applied by traditional users of sorghum biocolorant in West

* Corresponding author.
E-mail address: anita.linnemann@wur.nl (A.R. Linnemann).

https://doi.org/10.1016/j.lwt.2017.12.071
Received 20 July 2017; Received in revised form 27 December 2017; Accepted 28 December 2017
Available online 02 January 2018

Africa. This study evaluated (a) the degradation of sorghum total ACY in relation to heat treatments and storage conditions (light exposure, storage temperature), (b) the effect of a food acidulant (*viz*. citric acid) and alkaline conditions (NaOH) on apigeninidin solubility and degradation, (c) the thermal degradation kinetics and the resistance to bleaching of apigeninidin in relation to the pH of the watery extract and (d) the apigeninidin degradation and colour properties at different pH values in a food matrix, namely maize porridge.

2. Material and methods

2.1. Materials

Dried dye sorghum (*Sorghum bicolor*) leaf sheaths and *kanwu,* an alkaline rock containing carbonate and bicarbonate salts (Madodé, 2012) used by local users as an extraction aid, were bought at the market of Dassa-zoumè in Benin and ground into powder using a miller (Coffee Bean and Spice Mill Grinder Model #843, Moulinex). Maize grains (*Zea mays*) were purchased from the market of Abomey-Calavi in Benin, cleaned and milled into flour using a miller (RotorMill Pulvrisette-14, Idar-Oberstein, Germany) equipped with a 0.2 mm sieve as an ingredient for making maize porridge. Solutions of 1 N of citric acid (Sigma-Aldrich, Netherlands) and 1 N hydroxide sodium (Merck, Germany) were used as acidic and alkaline solutions, respectively.

2.2. Apigeninidin extraction procedures

Sorghum alkaline watery extract (SAWE) and sorghum hot aqueous extract (SHAE) were produced by cool alkaline and hot aqueous extraction, respectively (Akogou, Kayodé, den Besten, & Linnemann, 2017). These two watery extraction methods are the most common traditional extraction procedures in Benin. Alkaline extraction was conducted by mixing 11.1 g of sorghum leaf sheath powder, 1.5 g of *kanwu* and 1000 mL of water and stirring for 20 min at room temperature. For hot aqueous extraction, 11.1 g of sorghum leaf sheath powder and 1000 mL of water were mixed and heated with a magnetic heating plate (model FB 15010, Fischer Scientific) from 21.5 °C to 86 °C and then cooled down in an ice bucket. Both watery extracts were filtered with 2.5 µm filter paper (Whatman, GE Healthcare UK Limited, UK) to remove sorghum residues. The pH of the SAWE and the SHAE were 8.67 ± 0.14 and 7.07 ± 0.04, respectively.

2.3. Thermal treatment and storage of the watery extracts

Volumes of 6 mL of SAWE and SHAE were transferred in glass tubes and subjected to various heat treatments, i.e. 65 °C/30 min, 95 °C/30 min and 121 °C/30 min. A water bath (Memmert WNE 14, Schwabach, Germany) and an autoclave (Timo, Pbi International, Italy) were used to apply (a) 65 °C/30 min and 95 °C/30 min and (b) 121 °C/30 min, respectively. The water bath was preheated to 65 °C and 95 °C before applying the heat treatments. After the heat treatments, the tubes were cooled down in a cold water bath. Two independent duplicates of SAWE and SHAE were subjected to heat treatments. Next the stability of ACY to various storage conditions was evaluated by keeping independent duplicate extracts for 18 days (i) in the dark at room temperature (26–35 °C) (Adinsi et al., 2014), (ii) in the dark under refrigeration at 4 °C, and (iii) in ambient light at room temperature (26–35 °C) (Adinsi et al., 2014). Two tubes of SAWE and SHAE were taken (a) before and after treatment and (b) during storage for measurement of the total ACY content and the total colour density (TCD).

The total ACY content was measured using the pH differential method by Cao, Liu, Pan, Lu, and Xu (2008). The total ACY was measured only in the pH 1 buffer because the main ACY in sorghum extract (i.e. apigeninidin) is not colourless in a pH 4.5 buffer (Awika et al., 2004). Wavelengths of 470 and 700 nm were used for apigeninidin and haze correction, respectively. The total ACY content was calculated using the following Equation (1):

$$\text{Total ACY content}\left(\text{apigeninidin equivalent mg L}^{-1}\right) = \frac{A \times 290.69 \times DF \times 10^3}{\varepsilon_a \times l} \quad (1)$$

where $A = (A_{470\,nm} - A_{700\,nm})pH_{1.0}$; 290.69: molecular weight of apigeninidin chloride (g mol^{-1}); DF: dilution factor; 10^3: conversion factor from g to mg; ε_a: molar absorptivity of apigeninidin chloride (L mol^{-1} cm^{-1}); l: path length (which is 1 cm). The molar absorptivity of apigeninidin chloride was determined by the method of Cao et al. (2008) with an apigeninidin chloride standard (Extrasynthese, Genay, France).

The TCD was determined according to Turfan, Türkyılmaz, Yemiş, and Özkan (2011). Wavelengths of 420, 470 and 700 nm were used for brown pigments, apigeninidin and haze correction, respectively.

The TCD was calculated using Equation (2):

$$TCD = [(A_{420} - A_{700}) + (A_{470} - A_{700})]_{\text{non-treated sample}} \times DF \quad (2)$$

where DF: dilution factor.

The apigeninidin content and the TCD were measured using a Spectrophotometer (SmartSpec Plus spectrophotometer, Bio Rad, USA).

2.4. pH adjustment of the watery extract

Acidic (citric acid 1 N) and alkaline (hydroxide sodium 1 N) solutions were used to adjust the pH of independent duplicate samples of SAWE (50 mL, pH 8.67) to 5.04 ± 0.02, 6.08 ± 0.02, 10.03 ± 0.03, 11.04 ± 0.03 and 12.07 ± 0.04. Samples were poured in 50 mL polypropylene tubes (Cellstar, Greiner Bio-One, Frickenhausen, Germany), kept at room temperature for 30 min and centrifuged at 3000 rpm at 4 °C for 30 min with a centrifuge (Heraeus Multifuge X3R, Thermo Fisher Scientific, UK). Next, the supernatant was collected and the pellet was suspended in methanol. The plain extract was used when no pellet had been formed. The apigeninidin content, its molar adsorptivity and the formation of phenolic acids as degradation products in the supernatants, the in methanol suspended pellets and the plain extracts were determined by HPLC.

An ultimate 3000 RS High Performance Liquid Chromatography (HPLC) system equipped with a Diode Array Detector DAD-3000 RS (Thermo Scientific Dionex, Amsterdam, the Netherlands) and a quaternary pump LPG- 3000 RS (Thermo Scientific Dionex) was used. Standards of apigeninidin (Extrasynthese, France), 4-hydroxybenzoic acid (Sigma Aldrich, Netherlands) and *p*-coumaric acid (Sigma Aldrich, Netherlands) were used for identification and quantification. Compounds were separated with a Polaris C18-A column (150 × 4.6 mm, Varian, CA, USA) at a volumetric flow rate of 1 mL min^{-1} with two mobile phases, i.e. formic acid (10%) in milli-Q water (A) and methanol (100%) (B). The elution gradient of B was: 0–20 min, from 5% to 60% B; 20–25 min, from 60% to 100% B; 25–30 min with 100% B; 30–31 min from 100% to 5% B; 31–35 min with 5% B. Apigeninidin, 4-hydroxybenzoic acid and *p*-coumaric acid were measured at 480, 260 and 280 nm, respectively.

The molar absorptivity of apigeninidin was calculated using Equation (3) (Locatelli, Carlucci, Genovese, Curini, & Epifano, 2011):

$$\varepsilon_b = \frac{A \times f \times 255.24}{l \times c \times v \times 10^{-6}} \quad (3)$$

where ε_b: molar absorptivity (L mol^{-1} cm^{-1}); A: peak area recorded by HPLC (AU min); f: volumetric flow rate (L min^{-1}); 255.24: molecular weight of apigeninidin (g mol^{-1}); l: optical path length of the flow cell (which is 1 cm); c: concentration of apigeninidin (mg L^{-1}); 10^{-6}: conversion factor from mg L^{-1} to g mL^{-1}; v: volume injected (mL).

2.5. Degradation of apigenindin in watery extracts at different pH

Four independent replicates of SAWE were prepared. The pH was adjusted to 6.08 ± 0.02 and 9.03 ± 0.04 as described above, after which 6 mL samples were transferred to glass tubes and stored at 4 °C overnight (16 h). Next, the kinetic degradation of the extracts was performed at 65 °C using a block heater (Labtherm Liebisch, Bielefeld, Germany). Two tubes of SAWE at pH 6.08 ± 0.02 and 9.03 ± 0.04 were withdrawn at time intervals from 0 to 60 min to measure the apigeninidin content and the TCD as described above. In addition the polymeric colour (PC) and the % PC were determined on the watery extracts treated with bisulphite, see Equations (4) and (5) (Turfan et al., 2011). Wavelengths of 420, 470 and 700 nm were used for brown pigments, apigeninidin and haze correction, respectively. The experiment was performed four times with independent samples. The thermal degradation of apigeninidin was described with the natural logarithm scale using the Weibull model, see Equation (6).

$$PC = [(A_{420} - A_{700}) + (A_{470} - A_{700})]_{\text{treated sample}} \times DF \quad (4)$$

$$\%PC = \frac{PC}{TCD} \times 100 \quad (5)$$

$$\ln C_t = \ln C_0 - \left(\frac{t}{\delta}\right)^{\beta} \quad (6)$$

where, C_t: concentration of degraded apigeninidin (mg L^{-1}); C_0: initial apigeninidin content (mg L^{-1}); δ: the inverse of the kinectic rate constant (min^{-1}); β: shape constant, which defines the degree of concavity of the curve and t: time (min).

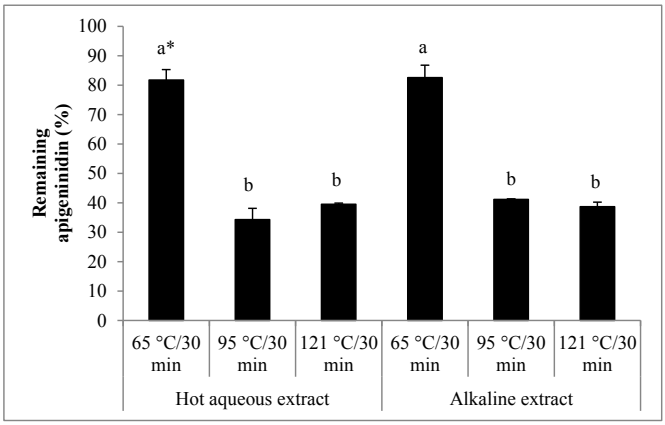
2.6. Combined effect of heat treatment and pH adjustment in a food matrix

Two independent replicates of SAWE and solution of *kanwu* (SK) (1.5 g L^{-1} in milli-Q water) were prepared and adjusted to pH 4.09 ± 0.19, 5.04 ± 0.02, 6.08 ± 0.02 and 9.03 ± 0.04 as described above. Maize flour (81 g) was added to 810 mL of the pH adjusted SAWE and SK. The mix was cooked using a thermomixer (Vorwerk, Wuppertal, Germany). The samples of maize porridge cooked with pH adjusted SK were used as control. The temperature of the cooking programme was set as follows: from 23 to 95 °C during the first 10 min and cooking at 95 °C during the next 10 min. Next, the samples were cooled down (i) at room temperature to measure colour and dry matter and (ii) with liquid nitrogen, freeze-dried at −55 °C/0.72 mbar (using an Alpha 1–4 LD plus freeze-dryer, Marin Christ, Germany) and milled by impact and friction (mixer mill MM400, Retsch, Haan, Germany) for extraction and quantification of apigeninidin. Colour parameters (L^*, a^*, b^*) were measured with a HunterLab Colorflex EZ spectrophotometer (Reston, VA, USA) (illuminant D 65 and 10° observer) and the chroma (C^*) and the hue ($h°$) were calculated.

2.7. Statistical analysis

Data on the total ACY, apigeninidin and phenolic acids (4-hydroxybenzoic acid and *p*-coumaric acid), TCD, PC, %PC, colour parameters (L^*, C^*, $h°$) were analysed with SPSS 23.0 (SPSS Inc, Chicago, IL, USA). One way variance analysis (ANOVA) on the means (or on the mean ranks when the normality or homogeneity of variance failed) followed by post hocs (Duncan or Mann-Whitney pairwise tests) were applied to detect differences between treatments (pH, heat treatment and time exposure).

The parameters of the Weibull model (ln C_0, δ and β) and their standard errors were estimated with the macro Solver Aid in Excel 2010. The f-value of the model was determined with Equation (7) (den Besten, Mataragas, Moezelaar, Abee, & Zwietering, 2006). The f-value was compared to the F-value table for α = 0.05 as shown in Equation (8) (den Besten et al., 2006).



*Bars with the same letters are not significantly different at 5%

Fig. 1. Stability of sorghum extracts to the most common heat treatments.

$$f\text{– value} = \frac{MSE_{model}}{MSE_{data}} \quad (7)$$

where, MSE_{model}: mean square error of the model; MSE_{data}: mean square error of the data

$$F^{DF_{model}}_{DF_{data}} = F^{n-s}_{n-m} \quad (8)$$

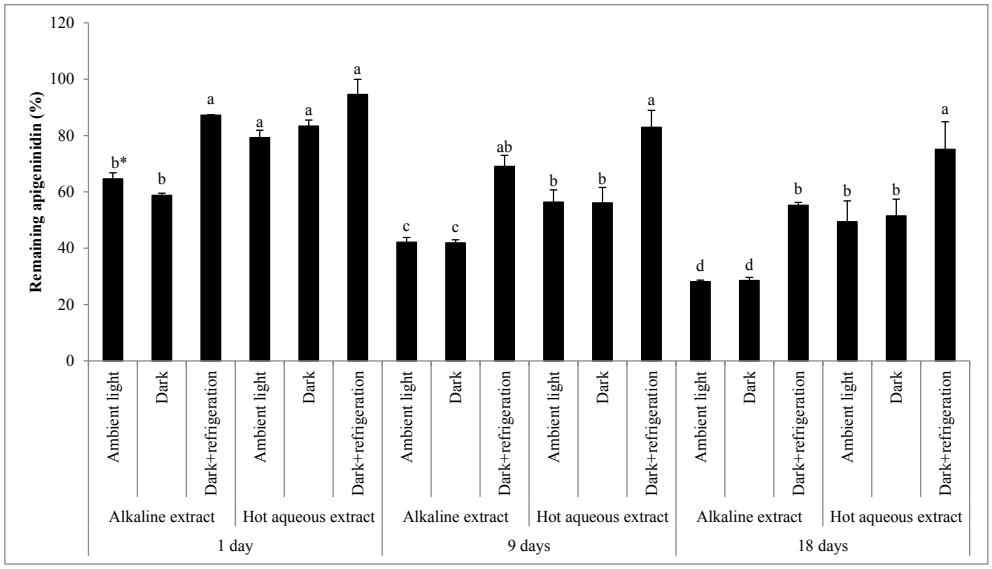
where DF_{model}: degrees of freedom of the model; DF_{data}: degrees of freedom of the data; n: number of data points, m: the number of time points; s: number of parameters of the model.

3. Results and discussion

3.1. Stability of total ACY in dye sorghum extract to heat treatment and storage conditions

Total ACY losses of 17–18%, 59–66% and 60–61% were measured after 65 °C/30 min, 95 °C/30 min and 121 °C/30 min heat treatments, respectively (Fig. 1). The percentage of total ACY that degraded due to the heat treatments was comparable for both types of watery extracts, although the total ACY differed (323.5 ± 11.8 mg L^{-1} and 181.0 ± 42.0 mg L^{-1} for SAWE and SHAE, respectively) as well as the pH values (8.67 ± 0.14 and 7.07 ± 0.04 for SAWE and SHAE, respectively). According to Brouillard, Iacobucci, and Sweeny (1982), the quinoidal bases are the dominant coloured forms of 3-deoxyanthocyanidins at pH 6. The pH of the SAWE and SHAE also suggested the quinoidal bases as the major forms of ACY at high pH (7.07–8.67). Apparently, the initial total ACY content and the shift from neutral to alkaline pH did not affect ($p = 0.4$ for 65 °C/30 min and $p = 0.1$ for 95 °C/30 min and 121 °C/30 min) the stability of ACY in relation to heat treatment. A previous study showed a loss of 80% of the anthocyanins at 100 °C/30 min (Hiemori, Koh, & Mitchell, 2009). In this respect, the ACY from sorghum extract performed much better at a neutral and alkaline pH. Data showed that sorghum extract can successfully be used in foods that require sterilisation treatments at pH 7.07 ± 0.04 and 8.67 ± 0.14, confirming the good thermal stability of apigeninidin (Yang et al., 2014) and providing additional information on thermal stability at alkaline pH.

Fig. 2 shows the stability of total ACY during storage. A general decrease of the apigeninidin content was observed during storage under ambient light and in the dark at room temperature (25–36 °C), as well as in the dark under refrigerated conditions (4 °C). After 18 days of storage, the decreases of total ACY in SAWE and SHAE were (a) 71.9% and 50.6% under ambient light, respectively, (b) 71.4% and 48.5% in the dark at room temperature, respectively and (c) 44.8% and 24.9% in the dark under refrigerated conditions, respectively. At room temperature, light conditions apparently had no influence on the apigeninidin decrease in SAWE or SHAE. Photodegradation of ACY could



*Bars with the same letters are not significantly different at 5%

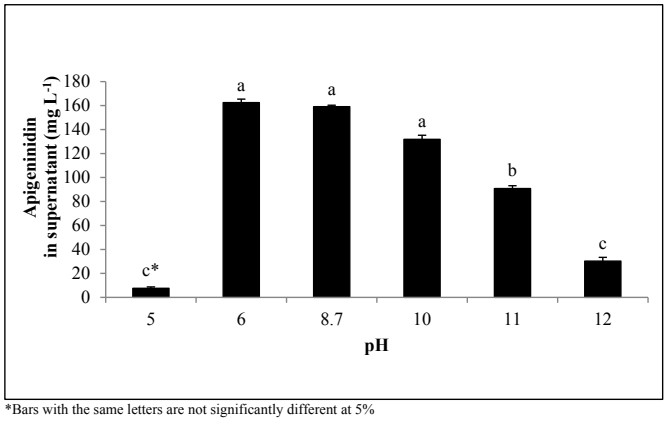
Fig. 2. Stability of pasteurised extracts to light and temperature during storage.

occur through the hydration of the flavylium cation into carbinol pseudobase (Dyrby et al., 2001). On the contrary, the high pH of SAWE and SHAE (8.67 ± 0.14 and 7.07 ± 0.04) promoted the formation of quinoidal base, which might not be involved in the photodegradation of ACY. Awika (2008) also reported good stability of flavylium cation of apigeninidin with only 25% loss after 15 days of storage under fluorescent light at 25 °C. The absence of increased degradation in extracts exposed to ambient light is advantageous since it implies that dye sorghum can be applied to foods and non-food products commonly exposed to light because common anthocyanins (i.e. glycosides of cyanidin and delphinidin) could lose more than 90% of the initial concentration of colorant after 15 days under light conditions (Baublis, Spomer, & Berber-Jiménez, 1994). The alkaline extract appeared to be particularly sensitive to storage at room temperature. Storage at room temperature (26–35 °C) increased the degradation of ACY due to its higher reactivity at high temperature (Alighourchi & Barzegar, 2009; Kırca & Cemeroğlu, 2003). Refrigerated storage slowed down the degradation of ACY. The reactions leading to the formation of the carbinol pseudobase and chalcone are known as endothermic (Brouillard, 1982). Therefore, storage at refrigeration temperature (4 °C) would retard the endothermic reactions leading to ACY degradation.

3.2. Effect of pH adjustment

The apigeninidin content of dye extracts was constant at pH 6–10 (Fig. 3), whereas its molar absorptivity did not change ($p = 0.06$) at pH 5–12 (27629.5 ± 85.7 L mol^{-1} cm^{-1}). Nevertheless, data reported in Table 1 shows that the use of an acidulant resulted in a lower TCD at pH 6.08 ± 0.02 than at pH 9.03 ± 0.04, implying that pH might affect the colour density of the watery extract of apigeninidin. A stable ACY molar absorptivity suggests a stable colour intensity (Torskangerpoll & Andersen, 2005). However, despite its stable molar absorptivity, the colour density of apigeninidin extract was not stable over the pH range tested. The phenolic acid content might affect the colour density of the apigeninidin watery extract as they could enhance the colour, contributing to a high TCD at high pH (Awika, 2008). Conversely, the oxidation of organic acids (e.g. citric acid) and their condensation with other phenolic compounds may occur at low pH values, contributing to a low colour intensity at pH 6.08 ± 0.02 (Kokkaew, Srithanyarat, & Pitirit, 2015).

The use of an acidulant at pH 5.04 ± 0.02 resulted in an apigeninidin precipitation with the formation of two phases: (i) a supernatant containing only 5% of the initial apigeninidin and (ii) a pellet rich in



*Bars with the same letters are not significantly different at 5%

Fig. 3. Effect of the pH adjustment on apigeninidin measured in solution at room temperature.

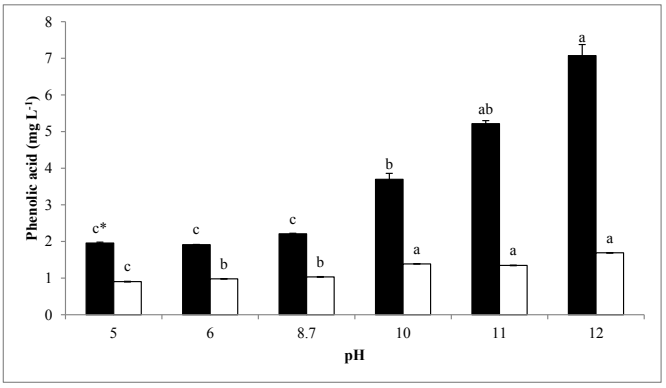
apigeninidin. HPLC analysis showed that the pellet contained apigeninidin with a purity of 100%. In addition, phenolic acids (viz., 4-hydroxybenzoic and *p*-coumaric acid) were absent at a detection limit of 1.95 µg mL^{-1}. Instead of the bleaching of apigeninidin in the presence of acidulants like acid ascorbic at pH 5 reported by Ojwang and Awika (2008), a loss of net charge leading to an apigeninidin precipitation in watery extract at pH 5 provided additional information on the effect of acid pH on apigeninidin extract. Precipitation of apigeninidin at pH 5.04 ± 0.02 might limit its application in acidic drinks (pH ≤ 5). On the contrary, an alkaline pH resulted in the degradation of apigeninidin. At pH 11.04 ± 0.03 and 12.07 ± 0.04, the apigeninidin concentration decreased by 44.1% and 81.4%, respectively, compared with the extract at pH 6.08 ± 0.02 (Fig. 3). According to literature, apigeninidin could be converted into phenolic acids at alkaline pH (Yang et al., 2014). Therefore alkaline extracts were analysed by HPLC. Parallel to the decrease of apigeninidin, an increase in the phenolic acid content of the extract was found (see Fig. 4). From pH 6.08 ± 0.02 to 12.07 ± 0.04, the concentrations of 4-hydroxybenzoic acid and *p*-coumaric acid increased 3.7 and 1.7 fold, respectively. Nevertheless, only 3.9 and 0.53% of the degraded apigeninidin were converted in 4-hydroxybenzoic acid and *p*-coumaric acid, respectively. This suggests that other phenolic acids might have been formed too.

The stability of apigeninidin and colour to heat treatment in a semisolid food matrix (i.e. a maize porridge) at different pH is reported

Table 1
Effect of heat treatment (65 °C) on total colour density and polymeric colour of sorghum biocolorant at pH 6.08 and 9.03.

Time (min)	pH 6.08			pH 9.03		
	TCD*	PC	%PC	TCD	PC	%PC
0	12.5 ± 1.6 a	3.9 ± 0.5 a	31.0 ± 0.2 a	45.4 ± 1.7 a	7.7 ± 0.4 a	17.0 ± 0.5 a
2.5	13.0 ± 1.7 a	4.2 ± 0.7 ab	32.4 ± 1.6 ab	44.8 ± 1.3 ab	7.9 ± 0.5 a	17.7 ± 0.7 ab
5	13.1 ± 2.2 a	4.2 ± 0.7 ab	31.8 ± 1.6 ab	44.5 ± 1.0 ab	8.1 ± 0.5 ab	18.2 ± 0.8 ab
10	12.9 ± 1.9 a	4.3 ± 0.6 ab	33.0 ± 0.9 b	44.2 ± 1.5 ab	8.2 ± 0.5 abc	18.5 ± 0.8 bc
20	13.6 ± 1.3 a	4.5 ± 0.5 ab	32.8 ± 1.1 b	43.0 ± 1.5 bc	8.4 ± 0.5 abc	19.6 ± 0.8 cd
30	12.9 ± 1.1 a	4.5 ± 0.3 ab	34.6 ± 0.8 c	42.6 ± 1.3 bc	8.5 ± 0.6 abc	20.0 ± 0.9 d
40	13.4 ± 1.6 a	4.7 ± 0.5 ab	34.9 ± 0.5 c	42.4 ± 1.6 bc	8.7 ± 0.7 bc	20.4 ± 1.0 de
50	12.6 ± 1.2 a	4.6 ± 0.4 ab	36.9 ± 0.1 d	42.0 ± 1.5 c	8.8 ± 0.6 abc	21.0 ± 0.9 de
60	12.6 ± 1.1 a	4.9 ± 0.4 b	38.7 ± 0.9 e	41.5 ± 1.7 c	9.0 ± 0.6 c	21.7 ± 0.9 e

*mean ± standard deviation; values with the same value in the same column are not significantly different at 5%.
TCD, total colour density; PC, polymeric colour; %PC, percentage of polymeric colour.



*Bars with the same colour and the same letters are not significantly different at 5%

Fig. 4. Effect of pH on the concentration of 4-hydroxydenzoic acid (■) and *p*-coumaric acid (□) in sorghum colorant.

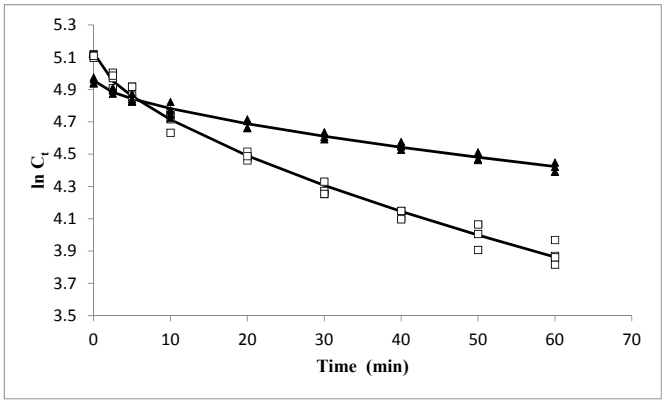


Fig. 5. Kinetic degradation at 65 °C of apigeninidin in dye sorghum extract at pH 6.08 (□) and 9.03 (▲) and the fitted data with using the Weibull model.

Table 2
Apigeninidin content and colour in cooked maize porridge.

pH	Apigeninidin content (mg/g DM)	*L**	*C**	*h*°
4.09 (n = 2)	1.3 ± 0.0 a	23.7 ± 0.3 b	33.3 ± 0.5 a	38.0 ± 0.8 b
5.04 (n = 2)	0.8 ± 0.0 a	24.2 ± 0.1 b	30.5 ± 0.1 a	34.8 ± 0.0 b
6.08 (n = 2)	0.8 ± 0.0 a	23.0 ± 0.3 b	28.8 ± 0.1 a	33.7 ± 0.3 b
9.03 (n = 2)	1.9 ± 0.2 b	13.6 ± 0.6 a	30.6 ± 0.3 a	27.9 ± 0.3 a

*mean ± standard deviation; values with the same value in the column are not significantly different at 5%.

Table 3
Values of parameters estimated with the Weibull model.

Extract pH	Estimated parameters	Mean	Standard error	f-value of the model*
6.08	ln C_0	5.12	0.02	1.0**
	δ	41.19	1.60	
	β	0.62	0.03	
9.03	ln C_0	4.96	0.01	0.9**
	δ	164.2	10.02	
	β	0.63	0.04	

*f-value = MSE_{model}/MSE_{data}.
**f-value < F-value table of the degrees of freedom of the model and the data (1.87) means Weibull model described the observed values well.

in Table 2. The apigeninidin content of the porridge did not differ at pH 4–6 ($p = 0.2$), whereas it was higher at pH 9.03 ± 0.04. Furthermore, the colour of the porridge was comparable at pH 4–6 whereas hue (*h*°) and lightness (*L**) were smaller at pH 9.03 ± 0.04. In other words, the porridge looked more red at pH 9.03 ± 0.04 than at pH 4–6. Consequently, the increased redness of the porridge and the higher concentration of apigeninidin at high pH might have resulted from (a) the higher TCD of the quinoidal forms of apigeninidin and (b) its higher resistance to heat treatment, respectively.

3.3. Kinetic degradation of sorghum biocolorant as related to pH

Fig. 5 shows the kinetic degradation of sorghum biocolorant at 65 °C and the fitted data. The degradation is lower at pH 9.03 ± 0.04 than at pH 6.08 ± 0.02. Table 3 summarizes the model parameters and their fitting performance (f-value). An f-value lower than the F-value table of the degrees of freedom of the model and the data support the proper fitting performance of the model. The stability of apigeninidin could adequately be described by three parameters, i.e. the natural logarithm of the initial concentration (ln C_0), the inverse of the kinetic rate constant (δ) and the shape parameter (β). The natural logarithm of the initial concentration (ln C_0) is higher at pH 6.08 ± 0.02 (5.12) than at pH 9.03 ± 0.04 (4.96). Moreover, the inverse of the kinectic rate constant (δ) is lower at pH 6.08 ± 0.02 (41.2) than at pH 9.03 ± 0.04 (164.2). Nevertheless, the shape parameter (β) was not different at pH 6.08 ± 0.02 (0.62) and pH 9.03 ± 0.04 (0.63). The pH change and the heat treatment affected the initial concentration and the kinetic rate constant. Although apigeninidin is stable at pH 6–9, its stability can apparently be affected by the time of exposure at alkaline pH. Indeed, storage at 4 °C overnight (i.e. for 18 h) affected ln C_0 at pH 9.03 ± 0.04. The lower degradation rate of apigeninidin at pH 9.03 ± 0.04 is in contrast to the degradation of most anthocyanins during heat treatment (Matsufuji et al., 2007). Few aglycone anthocyanins like apigeninidin showed such stability at high pH (Matsufuji

et al., 2007).

Table 1 shows the TCD, PC and %PC stability of the watery extract of sorghum biocolorant during a heat treatment (65 °C) at pH 6.08 ± 0.02 and 9.03 ± 0.04. The higher TCD and PC of quinoidal apigeninidin at pH 9.03 ± 0.04 compared to pH 6.08 ± 0.02 suggest (i) a higher colour intensity and (ii) a better resistance to bleaching, respectively. The higher colour density of the SAWE is used by processors to efficiently colour the surface of *wagashi,* a soft cheese of West Africa, at room temperature. During the heat treatment, the TCD was stable at pH 6.08 ± 0.02 whereas it decreased at pH 9.03 ± 0.04. In addition, the PC and the %PC of the extracts (at pH 6.08 and 9.03) increased during heating. Therefore, heat treatment (i) affected the density of the colour at pH 9.03 ± 0.04 and (ii) increased the amount of compounds resistant to bleaching at pH 6.08 ± 0.02 and 9.03 ± 0.04. The increased PC suggests the formation of new compounds reacting with sulphite. In addition, the chemical oxidation in heat treated polyphenol-containing foods could enhance the antioxidant activity (Nicoli, Anese, & Parpinel, 1999). Consequently, the increase of newly formed antioxidant reactive fragments might protect apigeninidin from the oxidizing activity of sulphite, leading to the increasing PC. More research is needed to identify the reactive fragments formed during heat treatment of apigeninidin extracts and how they enhance the resistance to bleaching.

3.4. Perspectives for the food industry

The alkaline treatment of sorghum leaf sheaths increased the release of the apigeninidin from the raw material and could thus be considered as an alternative and selective extraction method for 3-deoxyanthocyanidins (Akogou et al., 2017). The effect of the high pH on the apigeninidin concentration confirms extraction at pH around 8.7–9 to be the optimum to extract apigeninidin without the risk of degradation into phenolic acids. Considering the increased loss of apigeninidin at a decreasing pH using a heat treatment for food processing (Geera, Ojwang, & Awika, 2012), severe heat treatments should be applied in the pH range 7–9 to minimise loss of apigeninidin. The SAWE was more resistant to nucleophilic attack due to blanching.

4. Conclusion

Watery extracts from dye sorghum leaf sheaths demonstrated good stability (i) to processing conditions, including severe heat treatments, (ii) at pH 6–10 and (iii) to light exposure. Storage temperature affects stability; refrigerated conditions are to be preferred. Furthermore, the quinoidal base of apigeninidin (at pH 9) had a higher colour intensity and a better resistance to heat treatment. The loss of the net charge caused loss of solubility, leading to apigeninidin precipitation. Controlled acidification of alkaline extracts can be used to precipitate apigeninidin from watery extracts. Further research is needed to investigate (a) the control of the loss of net charge in acidic watery extracts of apigeninidin, (b) the identification of new antioxidant reactive fragments from apigeninidin degradation.

Acknowledgements

This research is funded by the Netherlands Organization for International Cooperation in Higher Education (Grant award CF8188/2012). The authors thank Matthijs Dekker for assistance with data analysis.

References

Adinsi, L., Vieira-Dalode, G., Akissoe, N., Anihouvi, V., Mestres, C., Jacobs, A., et al. (2014). Processing and quality attributes of gowé: A malted and fermented cereal-based beverage from Benin. *Food Chain, 4*(2), 171–183.

Akogou, F. U. G., Kayodé, A. P. P., den Besten, H. M. W., & Linnemann, A. R. (2017). Extraction methods and food uses of a natural red colorant from dye sorghum. *Journal of the Science of Food and Agriculture*. http://dx.doi.org/10.1002/jsfa.8479.

Alighourchi, H., & Barzegar, M. (2009). Some physicochemical characteristics and degradation kinetic of anthocyanin of reconstituted pomegranate juice during storage. *Journal of Food Engineering, 90*(2), 179–185.

Awika, J. M. (2008). Behavior of 3-deoxyanthocyanidins in the presence of phenolic copigments. *Food Research International, 41*(5), 532–538.

Awika, J. M., Rooney, L. W., & Waniska, R. D. (2004). Properties of 3-deoxyanthocyanins from sorghum. *Journal of Agricultural and Food Chemistry, 52*(14), 4388–4394.

Baublis, A., Spomer, A., & Berber-Jiménez, M. D. (1994). Anthocyanin pigments: Comparison of extract stability. *Journal of Food Science, 59*(6), 1219–1221.

den Besten, H. M. W., Mataragas, M., Moezelaar, R., Abee, T., & Zwietering, M. H. (2006). Quantification of the effects of salt stress and physiological state on thermotolerance of Bacillus cereus ATCC 10987 and ATCC 14579. *Applied and Environmental Microbiology, 72*(9), 5884–5894.

Brouillard, R. (1982). Chemical structure of anthocyanins. In P. Markakis (Ed.). *Anthocyanins as food colors*. Academic Press.

Brouillard, R., Iacobucci, G. A., & Sweeny, J. G. (1982). Chemistry of anthocyanin pigments. 9. UV-visible spectrophotometric determination of the acidity constants of apigeninidin and three related 3-deoxyflavylium salts. *Journal of the American Chemical Society, 104*(26), 7585–7590.

Cao, S., Liu, L., Pan, S., Lu, Q., & Xu, X. (2008). A comparison of two determination methods for studying degradation kinetics of the major anthocyanins from blood orange. *Journal of Agricultural and Food Chemistry, 57*(1), 245–249.

Dyrby, M., Westergaard, N., & Stapelfeldt, H. (2001). Light and heat sensitivity of red cabbage extract in soft drink model systems. *Food Chemistry, 72*(4), 431–437.

Geera, B., Ojwang, L. O., & Awika, J. M. (2012). New highly stable dimeric 3-deoxyanthocyanidin pigments from *Sorghum bicolor* leaf sheath. *Journal of Food Science, 77*(5), C566–C572.

Hiemori, M., Koh, E., & Mitchell, A. E. (2009). Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. *japonica* var. SBR). *Journal of Agricultural and Food Chemistry, 57*(5), 1908–1914.

Kayodé, A. P. P., Bara, C. A., Dalodé-Vieira, G., Linnemann, A. R., & Nout, M. J. R. (2012). Extraction of antioxidant pigments from dye sorghum leaf sheaths. *LWT-Food Science and Technology, 46*(1), 49–55.

Kokkaew, H., Srithanyarat, N., & Pitirit, T. (2015). Optimization of anthocyanin and effects of acidulants on phytochemicals and antioxidant activities in purple waxy corn cookies. *Asia-Pacific Journal of Science and Technology, 20*(1), 75–90.

Kırca, A., & Cemeroğlu, B. (2003). Degradation kinetics of anthocyanins in blood orange juice and concentrate. *Food Chemistry, 81*(4), 583–587.

Locatelli, M., Carlucci, G., Genovese, S., Curini, M., & Epifano, F. (2011). Use of HPLC in the determination of the molar absorptivity of 4′-geranyloxyferulic acid and boropinic acid. *Chromatographia, 73*(9–10), 889–896.

Madodé, Y. E. E. (2012). *Keeping local foods on the menu: A study on the small-scale processing of cowpea.* Wageningen, the Netherlands: Wageningen University.

Matsufuji, H., Kido, H., Misawa, H., Yaguchi, J., Otsuki, T., Chino, M., et al. (2007). Stability to light, heat, and hydrogen peroxide at different pH values and DPPH radical scavenging activity of acylated anthocyanins from red radish extract. *Journal of Agricultural and Food Chemistry, 55*(9), 3692–3701.

Mazza, G., & Brouillard, R. (1987). Recent developments in the stabilization of anthocyanins in food products. *Food Chemistry, 25*(3), 207–225.

Nicoli, M. C., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science & Technology, 10*(3), 94–100.

Ojwang, L., & Awika, J. M. (2008). Effect of pyruvic acid and ascorbic acid on stability of 3-deoxyanthocyanidins. *Journal of the Science of Food and Agriculture, 88*(11), 1987–1996.

Ojwang, L. O., & Awika, J. M. (2010). Stability of apigeninidin and its methoxylated derivatives in the presence of sulfites. *Journal of Agricultural and Food Chemistry, 58*(16), 9077–9082.

Torskangerpoll, K., & Andersen, Ø. M. (2005). Colour stability of anthocyanins in aqueous solutions at various pH values. *Food Chemistry, 89*(3), 427–440.

Turfan, Ö., Türkyılmaz, M., Yemiş, O., & Özkan, M. (2011). Anthocyanin and colour changes during processing of pomegranate (*Punica granatum* L., cv. Hicaznar) juice from sacs and whole fruit. *Food Chemistry, 129*(4), 1644–1651.

Wallace, T. C., & Giusti, M. M. (2015). Anthocyanins. *Advances in Nutrition: An International Review Journal, 6*(5), 620–622.

Yang, L., Dykes, L., & Awika, J. M. (2014). Thermal stability of 3-deoxyanthocyanidin pigments. *Food Chemistry, 160*, 246–254.