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

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15 **Abstract**

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

31

32 Keywords: Apigeninidin, heat treatment, pH, colour density, biocolorant

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

56 improves in the presence of phenolic acids (Awika, 2008). Most experiments on the stability of 3-
57 deoxyanthocyanidins were with non-food grade solvents such as ethanol, methanol,
58 hydroalcoholic solutions, water acidified with HCl or 70% aqueous acetone (Awika, 2008; Awika
59 et al., 2004; Kayodé, Bara, Dalodé-Vieira, Linnemann, & Nout, 2012; Ojwang & Awika, 2008).
60 Limited data exist on the stability of apigeninidin in watery extracts as commonly applied by
61 traditional users of sorghum biocolorant in West Africa. This study evaluated (a) the degradation
62 of sorghum total ACY in relation to heat treatments and storage conditions (light exposure, storage
63 temperature), (b) the effect of a food acidulant (*viz*. citric acid) and alkaline conditions (NaOH) on
64 apigeninidin solubility and degradation, (c) the thermal degradation kinetics and the resistance to
65 bleaching of apigeninidin in relation to the pH of the watery extract and (d) the apigeninidin
66 degradation and colour properties at different pH values in a food matrix, namely maize porridge.

67

68 **2. Material and methods**

69 2.1. Materials

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

78

79 ## 2.2. Apigeninidin extraction procedures

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

90

91 ## 2.3. Thermal treatment and storage of the watery extracts

92 Volumes of 6 mL of SAWE and SHAE were transferred in glass tubes and subjected to various
93 heat treatments, i.e. 65 °C / 30 min, 95 °C / 30 min and 121 °C / 30 min. A water bath (Memmert
94 WNE 14, Schwabach, Germany) and an autoclave (Timo, Pbi International, Italy) were used to
95 apply (a) 65 °C / 30 min and 95 °C / 30 min and (b) 121 °C / 30 min, respectively. The water bath
96 was preheated to 65 °C and 95 °C before applying the heat treatments. After the heat treatments,
97 the tubes were cooled down in a cold water bath. Two independent duplicates of SAWE and SHAE
98 were subjected to heat treatments. Next the stability of ACY to various storage conditions was
99 evaluated by keeping independent duplicate extracts for 18 days (i) in the dark at room temperature

100 (26-35 °C) (Adinsi et al., 2014), (ii) in the dark under refrigeration at 4 °C, and (iii) in ambient
101 light at room temperature (26-35 °C) (Adinsi et al., 2014). Two tubes of SAWE and SHAE were
102 taken (a) before and after treatment and (b) during storage for measurement of the total ACY
103 content and the total colour density (TCD).

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

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

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

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

117 The TCD was calculated using Equation (2):

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

118 where DF: dilution factor.

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

121

122 ## 2.4. pH adjustment of the watery extract

123 Acidic (citric acid 1 N) and alkaline (hydroxide sodium 1 N) solutions were used to adjust the pH
124 of independent duplicate samples of SAWE (50 mL, pH 8.67) to 5.04±0.02, 6.08±0.02,
125 10.03±0.03, 11.04±0.03 and 12.07±0.04. Samples were poured in 50 mL polypropylene tubes
126 (Cellstar, Greiner Bio-One, Frickenhausen, Germany), kept at room temperature for 30 min and
127 centrifuged at 3000 rpm at 4 °C for 30 min with a centrifuge (Heraeus Multifuge X3R, Thermo
128 Fisher Scientific, UK). Next, the supernatant was collected and the pellet was suspended in
129 methanol. The plain extract was used when no pellet had been formed. The apigeninidin content,
130 its molar adsorptivity and the formation of phenolic acids as degradation products in the
131 supernatants, the in methanol suspended pellets and the plain extracts were determined by HPLC.

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

141 31 to 35 min with 5% B. Apigeninidin, 4-hydroxybenzoic acid and *p*-coumaric acid were measured
142 at 480, 260 and 280 nm, respectively.

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

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

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

149

150 2.5. Degradation of apigenindin in watery extracts at different pH

151 Four independent replicates of SAWE were prepared. The pH was adjusted to 6.08±0.02 and
152 9.03±0.04 as described above, after which 6 mL samples were transferred to glass tubes and stored
153 at 4 °C overnight (16 hours). Next, the kinetic degradation of the extracts was performed at 65 °C
154 using a block heater (Labtherm Liebisch, Bielefeld, Germany). Two tubes of SAWE at pH
155 6.08±0.02 and 9.03±0.04 were withdrawn at time intervals from 0 to 60 min to measure the
156 apigeninidin content and the TCD as described above. In addition the polymeric colour (PC) and
157 the % PC were determined on the watery extracts treated with bisulphite, see Equations (4) and
158 (5) (Turfan et al., 2011). Wavelengths of 420, 470 and 700 nm were used for brown pigments,
159 apigeninidin and haze correction, respectively. The experiment was performed four times with

160 independent samples. The thermal degradation of apigeninidin was described with the natural
161 logarithm scale using the Weibull model, see Equation (6).

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

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

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

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

165

166 2.6. Combined effect of heat treatment and pH adjustment in a food matrix

167 Two independent replicates of SAWE and solution of *kanwu* (SK) (1.5 g L^{-1} in milli-Q water) were
168 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.
169 Maize flour (81 g) was added to 810 mL of the pH adjusted SAWE and SK. The mix was cooked
170 using a thermomixer (Vorwerk, Wuppertal, Germany). The samples of maize porridge cooked
171 with pH adjusted SK were used as control. The temperature of the cooking programme was set as
172 follows: from 23 to 95 °C during the first 10 min and cooking at 95 °C during the next 10 min.
173 Next, the samples were cooled down (i) at room temperature to measure colour and dry matter and
174 (ii) with liquid nitrogen, freeze-dried at -55 °C / 0.72 mbar (using an Alpha 1-4 LD plus freeze-
175 dryer, Marin Christ, Germany) and milled by impact and friction (mixer mill MM400, Retsch,
176 Haan, Germany) for extraction and quantification of apigeninidin. Colour parameters (L^*, a^*, b^*)

177 were measured with a HunterLab Colorflex EZ spectrophotometer (Reston, VA, USA) (illuminant

178 D 65 and 10° observer) and the chroma (C^*) and the hue ($h°$) were calculated.

179

180 2.8. Statistical analysis

181 Data on the total ACY, apigeninidin and phenolic acids (4-hydroxybenzoic acid and *p*-coumaric

182 acid), TCD, PC, %PC, colour parameters (L^*, C^*, $h°$) were analysed with SPSS 23.0 (SPSS Inc,

183 Chicago, IL, USA). One way variance analysis (ANOVA) on the means (or on the mean ranks

184 when the normality or homogeneity of variance failed) followed by post hocs (Duncan or Mann-

185 Whitney pairwise tests) were applied to detect differences between treatments (pH, heat treatment

186 and time exposure).

187 The parameters of the Weibull model (ln C_0, δ and β) and their standard errors were estimated with

188 the macro Solver Aid in Excel 2010. The f-value of the model was determined with Equation (7)

189 (den Besten, Mataragas, Moezelaar, Abee, & Zwietering, 2006). The f-value was compared to the

190 F-value table for α=0.05 as shown in Equation (8) (den Besten et al., 2006).

$$\mathrm{f-value} = \frac{\mathrm{MSE_{model}}}{\mathrm{MSE_{data}}} \quad (7)$$

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

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

192 where DF_{model}: degrees of freedom of the model; DF_{data}: degrees of freedom of the data; n: number

193 of data points, m: the number of time points; s: number of parameters of the model.

194

195 ## 3. Results and discussion

196 ### 3.1. Stability of total ACY in dye sorghum extract to heat treatment and storage conditions

197 Total ACY losses of 17-18%, 59-66% and 60-61% were measured after 65 °C / 30 min , 95 °C /
198 30 min and 121 °C / 30 min heat treatments, respectively (Fig. 1). The percentage of total ACY
199 that degraded due to the heat treatments was comparable for both types of watery extracts, although
200 the total ACY differed (323.5±11.8 mg L^{-1} and 181.0±42.0 mg L^{-1} for SAWE and SHAE,
201 respectively) as well as the pH values (8.67±0.14 and 7.07±0.04 for SAWE and SHAE,
202 respectively). According to Brouillard, Iacobucci, and Sweeny (1982), the quinoidal bases are the
203 dominant coloured forms of 3-deoxyanthocyanidins at pH 6. The pH of the SAWE and SHAE also
204 suggested the quinoidal bases as the major forms of ACY at high pH (7.07 to 8.67). Apparently,
205 the initial total ACY content and the shift from neutral to alkaline pH did not affect (p=0.4 for 65
206 °C / 30 min and p=0.1 for 95 °C / 30 min and 121 °C / 30 min) the stability of ACY in relation to
207 heat treatment. A previous study showed a loss of 80% of the anthocyanins at 100 °C / 30 min
208 (Hiemori, Koh, & Mitchell, 2009). In this respect, the ACY from sorghum extract performed much
209 better at a neutral and alkaline pH. Data showed that sorghum extract can successfully be used in
210 foods that require sterilisation treatments at pH 7.07±0.04 and 8.67±0.14, confirming the good
211 thermal stability of apigeninidin (Yang et al., 2014) and providing additional information on
212 thermal stability at alkaline pH.

213 Fig. 2 shows the stability of total ACY during storage. A general decrease of the apigeninidin
214 content was observed during storage under ambient light and in the dark at room temperature (25-
215 36 °C), as well as in the dark under refrigerated conditions (4 °C). After 18 days of storage, the
216 decreases of total ACY in SAWE and SHAE were (a) 71.9% and 50.6% under ambient light,
217 respectively, (b) 71.4% and 48.5% in the dark at room temperature, respectively and (c) 44.8%

218 and 24.9% in the dark under refrigerated conditions, respectively. At room temperature, light
219 conditions apparently had no influence on the apigeninidin decrease in SAWE or SHAE.
220 Photodegradation of ACY could occur through the hydration of the flavylium cation into carbinol
221 pseudobase (Dyrby et al., 2001). On the contrary, the high pH of SAWE and SHAE (8.67±0.14
222 and 7.07±0.04) promoted the formation of quinoidal base, which might not be involved in the
223 photodegradation of ACY. Awika (2008) also reported good stability of flavylium cation of
224 apigeninidin with only 25% loss after 15 days of storage under fluorescent light at 25 °C. The
225 absence of increased degradation in extracts exposed to ambient light is advantageous since it
226 implies that dye sorghum can be applied to foods and non-food products commonly exposed to
227 light because common anthocyanins (i.e. glycosides of cyanidin and delphinidin) could lose more
228 than 90% of the initial concentration of colorant after 15 days under light conditions (Baublis,
229 Spomer, & Berber-Jiménez, 1994). The alkaline extract appeared to be particularly sensitive to
230 storage at room temperature. Storage at room temperature (26-35 °C) increased the degradation of
231 ACY due to its higher reactivity at high temperature (Alighourchi & Barzegar, 2009; Kırca &
232 Cemeroğlu, 2003). Refrigerated storage slowed down the degradation of ACY. The reactions
233 leading to the formation of the carbinol pseudobase and chalcone are known as endothermic
234 (Brouillard, 1982). Therefore, storage at refrigeration temperature (4 °C) would retard the
235 endothermic reactions leading to ACY degradation.

236

237 ## 3.2. Effect of pH adjustment

238 The apigeninidin content of dye extracts was constant at pH 6-10 (Fig. 3), whereas its molar
239 absorptivity did not change (p=0.06) at pH 5-12 (27629.5±85.7 L mol^{-1} cm^{-1}). Nevertheless, data
240 reported in Table 1 shows that the use of an acidulant resulted in a lower TCD at pH 6.08±0.02

241 than at pH 9.03±0.04, implying that pH might affect the colour density of the watery extract of
242 apigeninidin. A stable ACY molar absorptivity suggests a stable colour intensity (Torskangerpoll
243 & Andersen, 2005). However, despite its stable molar absorptivity, the colour density of
244 apigeninidin extract was not stable over the pH range tested. The phenolic acid content might affect
245 the colour density of the apigeninidin watery extract as they could enhance the colour, contributing
246 to a high TCD at high pH (Awika, 2008). Conversely, the oxidation of organic acids (e.g. citric
247 acid) and their condensation with other phenolic compounds may occur at low pH values,
248 contributing to a low colour intensity at pH 6.08±0.02 (Kokkaew, Srithanyarat, & Pitirit, 2015).

249 The use of an acidulant at pH 5.04±0.02 resulted in an apigeninidin precipitation with the
250 formation of two phases: (i) a supernatant containing only 5% of the initial apigeninidin and (ii) a
251 pellet rich in apigeninidin. HPLC analysis showed that the pellet contained apigeninidin with a
252 purity of 100%. In addition, phenolic acids (viz., 4-hydroxybenzoic and *p*-coumaric acid) were
253 absent at a detection limit of 1.95 $\mu g\ mL^{-1}$. Instead of the bleaching of apigeninidin in the presence
254 of acidulants like acid ascorbic at pH 5 reported by Ojwang and Awika (2008), a loss of net charge
255 leading to an apigeninidin precipitation in watery extract at pH 5 provided additional information
256 on the effect of acid pH on apigeninidin extract. Precipitation of apigeninidin at pH 5.04±0.02
257 might limit its application in acidic drinks ($pH \leq 5$). On the contrary, an alkaline pH resulted in the
258 degradation of apigeninidin. At pH 11.04±0.03 and 12.07±0.04, the apigeninidin concentration
259 decreased by 44.1% and 81.4%, respectively, compared with the extract at pH 6.08±0.02 (Fig. 3).
260 According to literature, apigeninidin could be converted into phenolic acids at alkaline pH (Yang
261 et al., 2014). Therefore alkaline extracts were analysed by HPLC. Parallel to the decrease of
262 apigeninidin, an increase in the phenolic acid content of the extract was found. From pH 6.08±0.02
263 to 12.07±0.04, the concentrations of 4-hydroxybenzoic acid and *p*-coumaric acid increased 3.7 and

264 1.7 fold, respectively. Nevertheless, only 3.9 and 0.53% of the degraded apigeninidin were
265 converted in 4-hydroxybenzoic acid and *p*-coumaric acid, respectively. This suggests that other
266 phenolic acids might have been formed too.

267 The stability of apigeninidin and colour to heat treatment in a semisolid food matrix (i.e. a maize
268 porridge) at different pH is reported in Table 2. The apigeninidin content of the porridge did not
269 differ at pH 4-6 ($p=0.2$), whereas it was higher at pH 9.03±0.04. Furthermore, the colour of the
270 porridge was comparable at pH 4-6 whereas hue ($h°$) and lightness (L^*) were smaller at pH
271 9.03±0.04. In other words, the porridge looked more red at pH 9.03±0.04 than at pH 4-6.
272 Consequently, the increased redness of the porridge and the higher concentration of apigeninidin
273 at high pH might have resulted from (a) the higher TCD of the quinoidal forms of apigeninidin
274 and (b) its higher resistance to heat treatment, respectively.

275

276 3.3. Kinetic degradation of sorghum biocolorant as related to pH

277 Fig. 5 shows the kinetic degradation of sorghum biocolorant at 65 °C and the fitted data. The
278 degradation is lower at pH 9.03±0.04 than at pH 6.08±0.02. Table 3 summarizes the model
279 parameters and their fitting performance (f-value). An f-value lower than the F-value table of the
280 degrees of freedom of the model and the data support the proper fitting performance of the model.
281 The stability of apigeninidin could adequately be described by three parameters, i.e. the natural
282 logarithm of the initial concentration ($\ln C_0$), the inverse of the kinetic rate constant (δ) and the
283 shape parameter (β). The natural logarithm of the initial concentration ($\ln C_0$) is higher at pH
284 6.08±0.02 (5.12) than at pH 9.03±0.04 (4.96). Moreover, the inverse of the kinectic rate constant
285 (δ) is lower at pH 6.08±0.02 (41.2) than at pH 9.03±0.04 (164.2). Nevertheless, the shape

286 parameter (β) was not different at pH 6.08±0.02 (0.62) and pH 9.03±0.04 (0.63). The pH change
287 and the heat treatment affected the initial concentration and the kinetic rate constant. Although
288 apigeninidin is stable at pH 6-9, its stability can apparently be affected by the time of exposure at
289 alkaline pH. Indeed, storage at 4 °C overnight (i.e. for 18h) affected ln C_0 at pH 9.03±0.04. The
290 lower degradation rate of apigeninidin at pH 9.03±0.04 is in contrast to the degradation of most
291 anthocyanins during heat treatment (Matsufuji et al., 2007). Few aglycone anthocyanins like
292 apigeninidin showed such stability at high pH (Matsufuji et al., 2007).

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

308

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.

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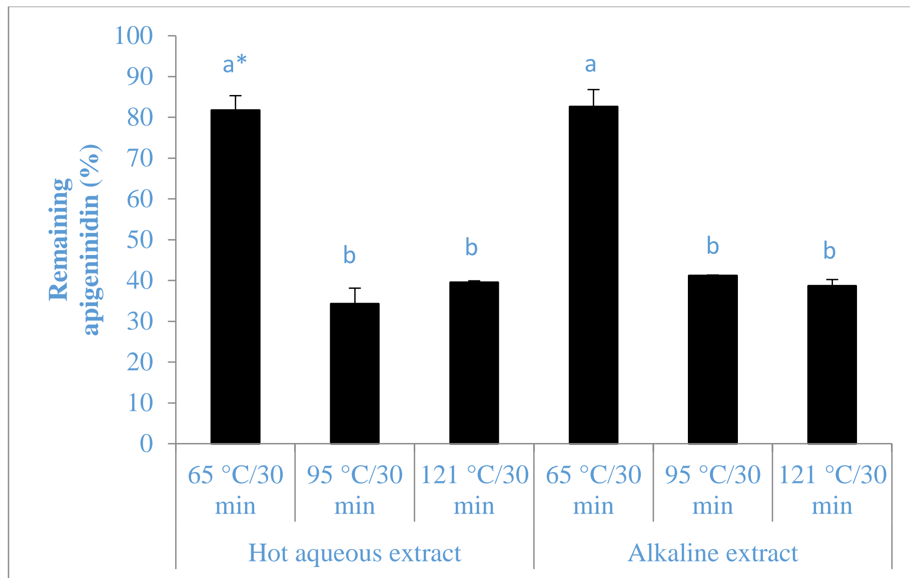

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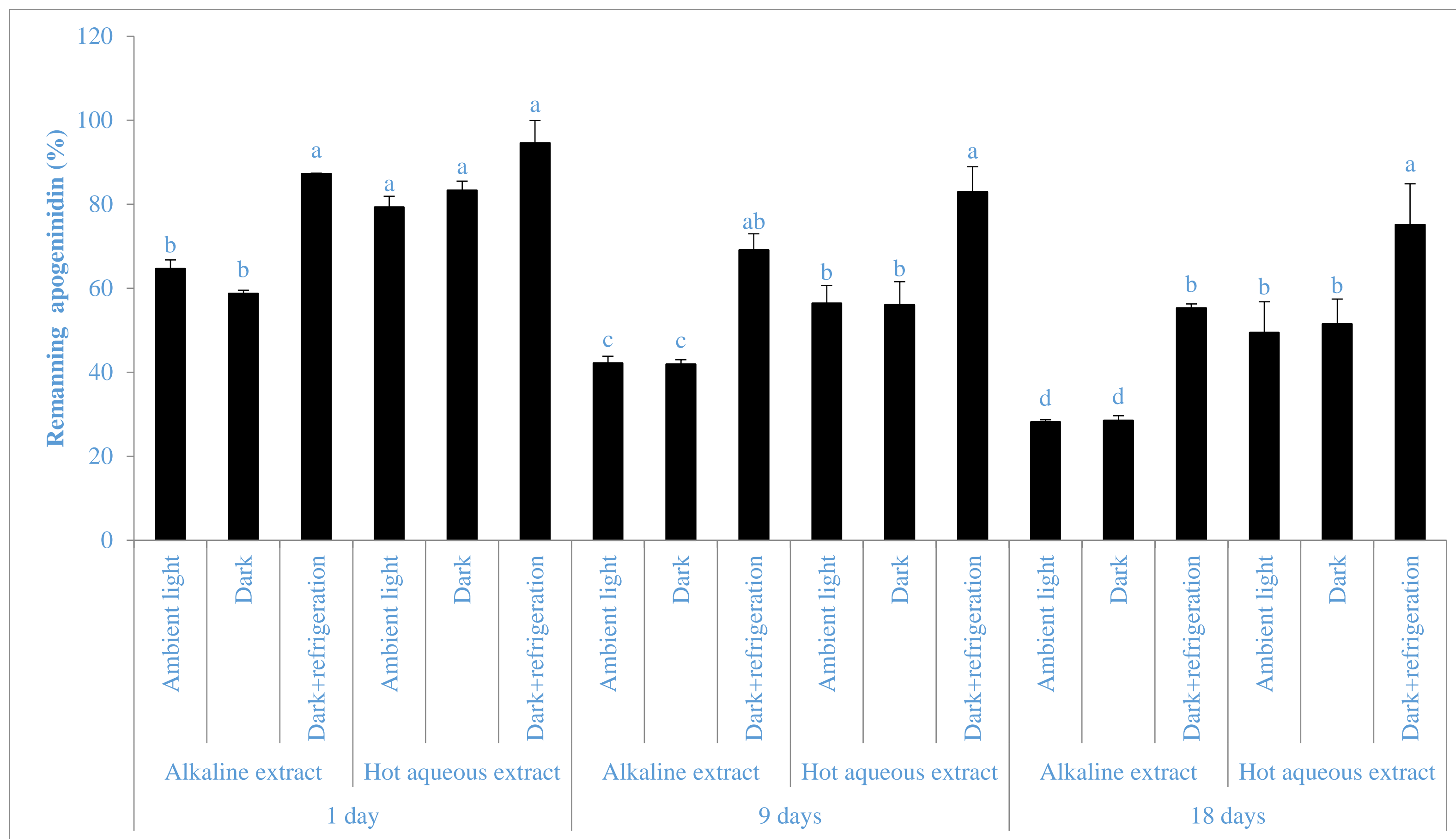
410 *Bars with the same letters are not significantly different at 5%

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

412



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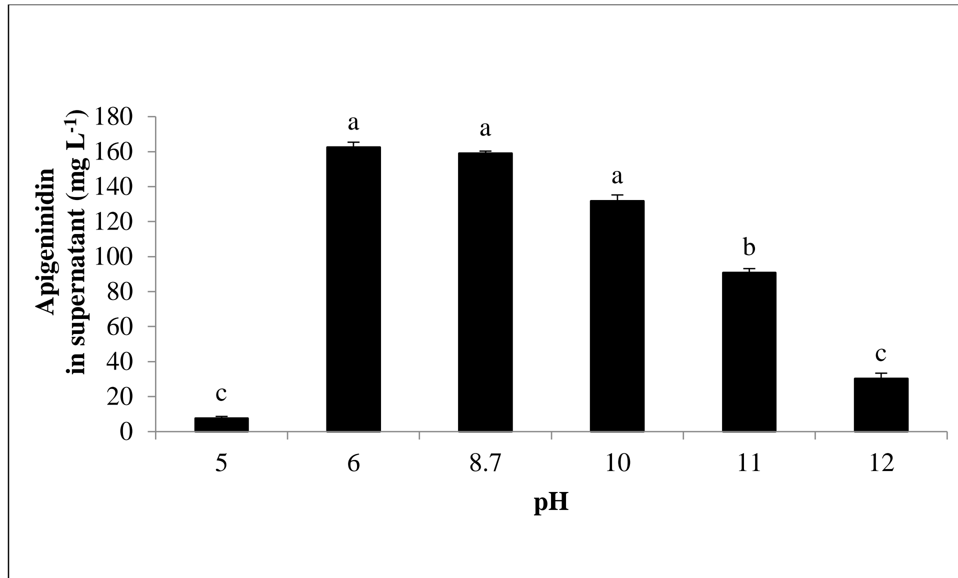
414 *Bars with the same letters are not significantly different at 5%

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

416



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

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

421

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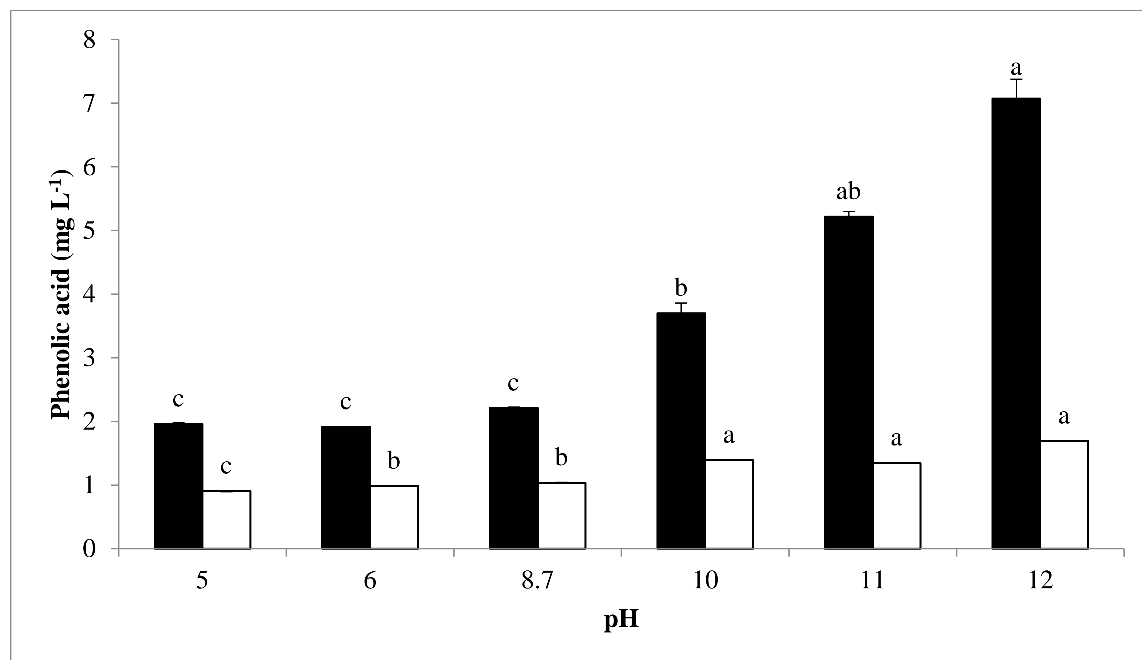

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424 *Bars with the same colour and the same letters are not significantly different at 5%

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

426 in sorghum colorant.

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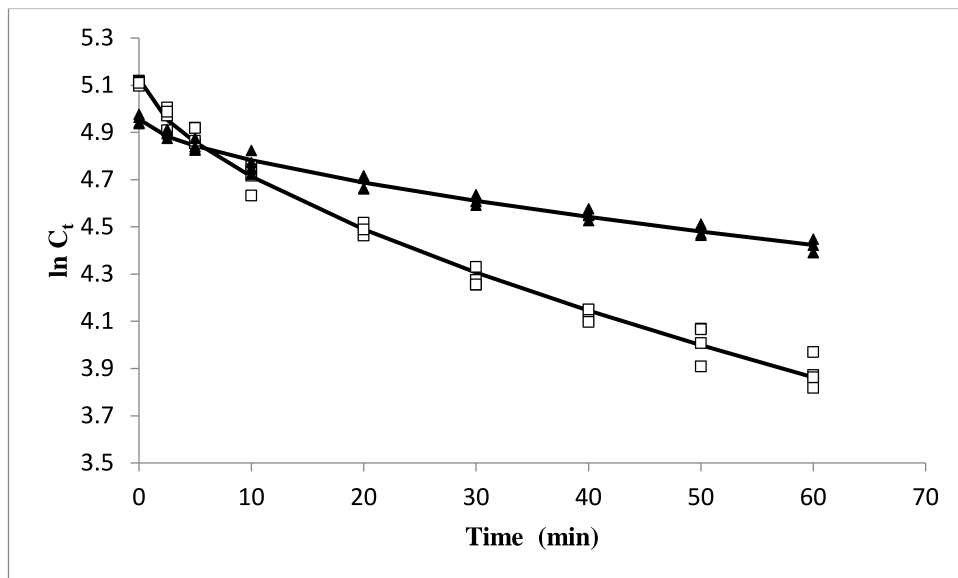

428

429 **Fig. 5.** Kinetic degradation at 65 °C of apigeninidin in dye sorghum extract at pH 6.08 (□) and

430 9.03 (▲) and the fitted data with using the Weibull model.

431 **Table 1: Effect of heat treatment (65 °C) on total colour density and polymeric colour of**
432 **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

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

434 TCD, total colour density; PC, polymeric colour; %PC, percentage of polymeric colour

435 **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±0.3 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

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

437

438 **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	

439 *f-value= MSE_{model}/MSE_{data}

440 **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