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Exploration of reaction mechanisms of anthocyanin degradation in a roselle extract through kinetic studies on formulated model media

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ABSTRACT

Effect of oxygen, polyphenols and metals was studied on degradation of delphinidin and cyanidin 3-*O*-sambubioside of *Hibiscus sabdariffa* L. Experiments were conducted on aqueous extracts degassed or not, an isolated polyphenolic fraction and extract-like model media, allowing the impact of the different constituents to be decoupled. All solutions were stored for 2 months at 37 °C. Anthocyanin and their degradation compounds were regularly HPLC-DAD-analyzed. Oxygen concentration did not impact the anthocyanin degradation rate. Degradation rate of delphinidin 3-*O*-sambubioside increased 6-fold when mixed with iron from 1 to 13 mg.kg^{-1} but decreased with chlorogenic and gallic acids. Degradation rate of cyanidin 3-*O*-sambubioside was not affected by polyphenols but increased by 3-fold with increasing iron concentration with a concomitant yield decrease of scission product, protocatechuic acid. Two pathways of degradation of anthocyanins were identified: a major metal-catalyzed oxidation followed by condensation and a minor scission which represents about 10% of degraded anthocyanins.

1. Introduction

Calyces of roselle, a tropical annual crop belonging to the *Malvaceae* family (*Hibiscus Sabdariffa* L.), are traditionally used to prepare a bright red beverage by decoction in water. Its strong red coloration is due to the main anthocyanins present in the calyces: delphinidin 3-*O*-sambubioside and cyaniding 3-*O*-sambubioside (Cisse, Dornier et al., 2009). Chemically, anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of flavylium salts. The intensity of the anthocyanin color is attributed to the resonant structure of the flavylium ion (Castaneda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Besides their vibrant colors, anthocyanins also have anti-oxidant and bioactive properties linked to certain health benefits *e.g.*, anti-diabetic, anti-inflammatory, and anti-cancer effects (Signorelli et al., 2015).

Several studies regarding the storage stability of anthocyanin pigments have been conducted in model beverages and juice extracts. The storage temperature was found to be the main factor responsible for anthocyanin loss (Cisse, Vaillant, Acosta, Dhuique-Mayer, & Dornier, 2009; Jiménez et al., 2010). The degradation rate is affected by several factors including pH, temperature, oxygen, metals ions, and other polyphenols (Beñitez, Castro, Jose Antonio Sanchez, & Barroso, 2002; Bordenave, Hamaker, & Ferruzzi, 2014; Chung, Rojanasasithara, Mutilangi, & McClements, 2016). The knowledge of the influence of some of these endogenous factors may help to understand the mechanism involved in the degradation of anthocyanins from hibiscus. In previous studies, the effect of temperature on degradation of the main anthocyanins from hibiscus was studied (Sinela et al., 2017). However, complex composition of extract did not allow the factors that affect the rates of degradation of anthocyanins to be identified because they can be multiple. Working on extract and at the same time on model media could be a good alternative to decouple the effect of each constituent assumed to have an impact on anthocyanin degradation. This approach seemed to be a good compromise to conduct a kinetic study, simplifying the medium composition while enabling comparison with the initial matrix (Hadjal, Dhuique-Mayer, Madani, Dornier, & Achir, 2013).

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Among the different factors known to affect degradation of anthocyanins, we focused in this study on oxygen, metals, phenolic acids and aldehydes because there is no information about their effects on degradation kinetics of anthocyanins from roselle. The mechanism involved on degradation of these anthocyanins was also explored in the light of a kinetic approach.

2. Materials and methods

2.1. Plant material

Calyces of *Hibiscus sabdariffa* L. used belonged to the variety Vimto, cultivated in the village of Thiaré, Senegal. Upon harvesting, the calyces were manually shelled and sun-dried on racks for 5 to 10 days.

2.2. Chemicals

Delphinidin 3-*O*-sambubioside (Del-3Sb), cyanidin 3-*O*-sambubioside (Cya-3Sb), 3-caffeoylquinic acid (CA), protocatechuic acid, gallic acid and malic acid were obtained from Extrasynthèse (Genay, France). Iron chloride, copper chloride, manganese chloride, and Amberlite XAD-16 resin were from Sigma Aldrich (L'Isle d'Abeau, France). All solvents were of HPLC grade and purchased from Carlo Erba (Val de Reuil, France). Deionized water was produced by a Milli-Q unit (Millipore, Bedford, MA, USA).

2.3. Preparation of the extract and polyphenolic fraction

Calyces were first ground with an electric grinder (Vorwerk Thermomix, France) and macerated in deionized water using a calyces/water ratio of 1/10 (w/w) for 30 min at 25 °C. Manual agitation was carried out regularly. The mix was then filtered through a filter bag (10 × 38 cm, 25 μm mesh, Leentech, Belgium) to obtain the crude extract. To evaluate the oxygen impact, this extract was deaerated by nitrogen stripping until the dissolved oxygen content decreased below 0.5 mg.L^{-1}. Oxygen content was measured using Multi 350i oxymeter (WTW, Germany).

For phenolic isolation, fractionation of the extract was carried out on a column (22 cm × 2.5 cm) filled with Amberlite XAD-16 resin, previously conditioned with 4 equivalent volumes of solvated resin (bed volume BV) with methanol/water (80/20 v/v), 4 BV of methanol/water (20/80 v/v) and 4 BV of milli-Q water. After loading the extract (2 mL) on the resin, the column was washed with 4 BV of milli-Q water then eluted with 4 BV methanol/water (80/20 v/v) and finally with 4 BV of pure methanol at a flow rate of 1 mL.min^{-1}. The eluted fractions were combined, evaporated to dryness under vacuum and then redissolved with 2 mL milli-Q water (equivalent volume of extract adsorbed on the resin). The obtained fraction was called the polyphenolic fraction (PF). Its pH was adjusted to the pH of the extract with a few drops of concentrated hydrochloric acid (12 M).

The crude extract and polyphenolic fraction were finally placed in 15 mL amber vials that were hermetically sealed. Because the crude extract was highly fermentable, microbiological stabilization was required. According to the low pH of the product, a classical thermal pasteurization was selected. So the extract was treated at 84 °C for 2 min which corresponds to a pasteurization value of 50 min as generally applied in fruit juice processing industry (calculated with z = 10 °C and 70 °C as reference temperature). The polyphenolic fraction was treated in the same way in order to easy comparison. In these conditions, no significant impact on the composition was detected (Sinela et al., 2017).

2.4. Preparation of model media

All media solutions were prepared in 0.1 M malic acid solution (pH = 2.2) whose compositions are shown in Table 1. To better assess the effect of CA on both anthocyanins, the same concentration of CA was used. For degradation products we used the same concentration for the same reason, in order to be sure that effect was not underestimated by a lack of co-reactant.

2.5. Storage of extract, polyphenolic fraction and media solutions

Extract and polyphenolic fraction and media solutions were stored in amber vials at the isothermal condition of 37 °C. A temperature of 37 °C was chosen according to Sinela et al. (2017). This high temperature allowed to obtain exploitable kinetic in the reasonable time of 60 days.

2.6. Analyses

2.6.1. Metals

Dosage method of metals present in the extract and polyphenolic fraction was adapted from the international norm NF EN ISO 11885 for metals quantification in water. Samples were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (VARIAN VISTA, Victoria, Australia), equipped with a Coupled Charged Device detector.

2.6.2. Phenolic compounds

Phenolics were analyzed by HPLC-DAD (Sinela et al., 2017). All the samples (20 μL injection volume) were filtered before (Millipore, 0.45 μm) and then analyzed on an HPLC DIONEX ULTIMATE 3000 equipped with a diode array detector and an autosampler. A reversed phase ACEC18 column (250 mm × 4.6 mm, 5 μm, AIT, France) thermostated at 30 °C was used for the separation. The mobile phase was constituted of water/formic acid/acetonitrile

Table 1
Formulation of model media (concentrations in mg.L^{-1}).

Compounds	Model media n°																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Delphinidin 3-O-sambubioside	–	–	–	200	–	200	–	200	–	–	200	200	–	–	–	–	–	200	–	–	–
Gallic acid	–	–	–	–	–	–	–	–	–	–	200	–	–	–	–	–	–	–	200	–	200
Protocatechuic acid																				200	200
Cyanidin 3-O-sambubioside	200	200	200	–	200	–	200	–	200	200	–	200	200	200	200	200	200	–	–	–	–
Phloroglucinaldehyde	–		–	–	–	–	–	–	–	200–	–	–	–	–	–	–	–	–	–		
Caffeoyl-3-quinic acid	–	200	–	–	200	200	200	200	200	–	–	200	–	200	200	–	200	200	–	–	–
Fe^{3+} ($FeCl_3$ used)	–		13	13	1	1	7	13	13	13	13	13	–	–	1	1	1	1	13	13	13
Cu^{2+} ($CuCl_2$ used)	–		–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.3	0.3	0.3	–	–	–
Mn^{2+} ($MnCl_2$ used)	–		–	–	–	–	–	–	–	–	–	–	20	20	–	20	20	20	–	–	–