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# Anthocyanins degradation during storage of *Hibiscus sabdariffa* extract and evolution of its degradation products

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ABSTRACT

Degradation parameters of two main anthocyanins from roselle extract (*Hibiscus sabdariffa* L.) stored at different temperatures (4–37 °C) over 60 days were determined. Anthocyanins and some of their degradation products were monitored and quantified using HPLC–MS and DAD. Degradation of anthocyanins followed first-order kinetics and reaction rate constants (k values), which were obtained by non-linear regression, showed that the degradation rate of delphinidin 3-O-sambubioside was higher than that of cyanidin 3-O-sambubioside with k values of $9.2{\cdot}10^{-7}\ s^{-1}$ and $8.4{\cdot}10^{-7}\ s^{-1}$ at 37 °C respectively. The temperature dependence of the rate of anthocyanin degradation was modeled by the Arrhenius equation. Degradation of delphinidin 3-O-sambubioside (Ea = 90 kJ $mol^{-1}$) tended to be significantly more sensitive to an increase in temperature than cyanidin 3-O-sambubioside (Ea = 80 kJ $mol^{-1}$). Degradation of these anthocyanins formed scission products (gallic and protocatechuic acids respectively) and was accompanied by an increase in polymeric color index.



## 1. Introduction

Anthocyanins represent the largest group of water soluble pigments in plants. They are highly appreciated in the food industry for their coloring properties, which can give food various hues of red and violet. Many edible plants are sources of anthocyanins, these include roselle calyces (*Hibiscus sabdariffa*) (Cisse et al., 2009; Du & Francis, 1973; Wong, Yusof, Ghazali, & Che Man, 2002).

*Hibiscus sabdariffa* is an herbaceous plant, cultivated largely in tropical and subtropical areas of both hemispheres. Its calyces (consumed in large quantities in Africa and Asia as a beverage following maceration in water) contain high amounts of anthocyanins, especially delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside, up to 2.5 g/100 g DM (Du & Francis, 1973; Juliani et al., 2009; Wong et al., 2002).

Recently, research carried out on anthocyanins has drawn much attention as they do not only impart beautiful coloration to food products but also have antioxidant properties and health benefits such as enhancement of sight acuteness, antioxidant capacity, controlling Type II diabetes, reduction of coronary heart disease and prevention of cancer (Peleg, Kim, & Normand, 2015; Thilakarathna & Rupasinghe, 2013).

Heat processing (e.g., pasteurization and sterilisation) is an extremely common and effective method of preserving beverages but may result in quality loss (Kırca & Cemeroglu, 2003). Indeed, elevated temperatures can affect anthocyanin stability and cause monomeric anthocyanins to polymerize, resulting in browning, which is undesirable in products such as fruit juices because consumers perceive it as an indication of inferior quality (Kirca & Cemeroglu, 2003; Somers, 1968). A lot of studies have reported that degradation of anthocyanins, at relevant temperatures (up to 100 °C) in food processing or during storage follows first-order kinetics (Ahmed, Shivhare, & Raghavan, 2004; Cemeroglu, Velioglu, & Isik, 1994; Kirca & Cemeroglu, 2003; Patras, Brunton, O'Donnell, & Tiwarib, 2010).

The aim of this study is to independently determine the kinetic parameters for delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside during calyces extract storage at various temperatures as well as analyzing changes in color and extract composition from a kinetic and mechanistic point of view. This will be helpful to limit anthocyanins degradation in Hibiscus extract during storage.

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## 2. Materials and methods

### 2.1. Plant material

Calyces of Hibiscus *sabdariffa* used in the experiments described hereafter 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 5–10 days.

### 2.2. Reagents

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). The polyphenols were quantified using standards (delphinidin 3-O sambubioside, cyanidin 3-O-sambubioside, 3-caffeoylquinic acid, gallic, and protocatechuic acids, quercetin, and kaempferol) supplied by Extrasynthese (Genay, France).

### 2.3. Extract preparation, thermal treatment and storage

To prepare the roselle extract, calyces were 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 ambient temperature. Manual agitation was carried out regularly. The mix was then successively filtered through a filter bag (10 × 38 cm, pore diameter 25 µm, Leentech, Belgium) and placed in amber vials that were hermetically sealed, containing 15 mL of extract. The pH of the extract was 2.2. The vials where submitted to a pasteurization treatment carried out by immersion in an oilbath under temperature control. A digital temperature probe (Almemo® ZA9020-FS thermo E4, France) fitted to a reference tube was used to measure the juice temperature during the thermal experiments. The couple temperature/time used was 84 °C/2 min leading to a target pasteurization value of 50 min calculated at a reference temperature of 70 °C with z = 10 °C.

After thermal treatment, the amber vials were stored under controlled temperatures of 4, 20, 30 and 37 °C for 60 days.

### 2.4. Identification and quantification of polyphenol compounds

For polyphenol identification, 1 g of calyx powder of *Hibiscus sabdariffa* was added to 30 mL of a solution of acetone/water/formic acid (70/28/2 v/v/v). The mixture was agitated for 10 min and then filtered through a paper filter. The filtrate was evaporated to dryness under vacuum and dissolved in 20 mL of a methanol/water mixture (50/50v/v). The solution was filtered (Millipore, 0.45 µm) prior to analysis by HPLC–MS.

Characterization was performed on an HPLC chain SURVEYOR, equipped with a diode array detector model UV6000LP, a quaternary pump P4000, an auto sampler AS3000 and coupled to a mass spectrometer LCQ equipped with an electro-spray ionization source (THERMO FINNIGAN, SanJose, USA). A column ACEC18 (250 mm × 4.6 mm, 5 µm, AIT, France) thermostated at 30 °C was used. The mobile phase was constituted of water/formic acid/acetonitrile (99.1/0.1/0.8 v/v/v) as solvent A and acetonitrile as solvent B. Twenty µL of solution was injected. The flow rate was 0.7 mL $min^{-1}$ with a linear gradient from 5 to 25% of solvent B in 50 min and then to 100% of solvent B in 2 min. Eluted compounds were detected at 280, 330, 360 and 510 nm. The electro-spray was performed in the negative mode. The mass range was between 100 and 2000 Da. The desolvation temperature was 300 °C. The spray tension was 5000V. The fragmentation of the type $MS^n$ was performed with collision energy between 30 and 50%.

Anthocyanins, flavonols, as well as chlorogenic, gallic, and protocatechuic acids were quantified by HPLC DIONEX ULTIMATE 3000 equipped with a diode array detector. The same operating conditions as for identification were used but the solvent A was modified to 97.2/2/0.8 (v/v/v) of water/formic acid/acetonitrile. Concentrations were determined using a calibration curve of corresponding standards and the measurement was carried out in triplicate.

### 2.5. Kinetic modeling of anthocyanin degradation

The isothermal degradation of anthocyanins was predicted using a first order model (Eq. (1)).

$$\frac{C}{C_0} = e^{-kt} \tag{1}$$

where $C_0$ and C are the initial concentration of anthocyanins and at time t (mol $L^{-1}$), k the rate constant ($s^{-1}$) and t the time (s).

The kinetic constant was identified by nonlinear regression with a least square minimization procedure using the complement Excel "Solver". This procedure allows a more accurate identification of constants compared to the usual logarithm linearization (Van Boekel, 2009). Uncertainty of the rate constants was obtained by the VBE Macro "SolverAid" (de Lavie, 2012).

The activation energy and the rate constant at the temperature of reference were determined according to the reparametrized Arrhenius equation (Eq. (2)).

$$\frac{k}{k_{ref}} = \exp\left[\frac{-Ea}{R}*\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right] \tag{2}$$

where k is the rate constant ($s^{-1}$), $k_{ref}$ the rate constant at the reference temperature ($s^{-1}$), Ea the activation energy (J $mol^{-1}$), R the perfect gas constant (8.32 J $mol^{-1}$ $K^{-1}$), T the temperature (K) and $T_{ref}$ the reference temperature chosen as the average storage temperature (293.13 K).

### 2.6. Spectrophotometric measurements

Brown index was determined by spectrophotometric measurements (spectrophotometer SPECORD S600, Analytik Jena, Germany) after dilution of the extract in malic acid at a pH of 2.2 (1/90 v/v). It was calculated according to Eq. (3).

$$BI = \frac{A_{(420nm)}}{A_{(520nm)}} \tag{3}$$

where A is the absorbance obtained at a specific wavelength.

Polymeric color index is the ratio of the polymeric color on the color density (Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999). A metabisulfite solution was added to sample solutions in order to discolor the anthocyanin monomers. The residual color (called polymeric color) of the sample solutions is attributed to the presence of polymeric materials. Samples were diluted in a malic acid solution at a pH of 2.2 (1/90 v/v). 2.8 mL of diluted samples were added to 0.2 mL of milli-Q water or 0.2 mL of metabisulfite (1 M) for measuring the color density and the polymeric color respectively. Polymer color index was determined by employing the Eqs. (4)–(6).

$$\textbf{Polymeric color index} = \frac{\textbf{Polymeric color}}{\textbf{Color density}} \tag{4}$$

$$\textbf{Polymeric color} = [(\mathbf{A_{420}} - \mathbf{A_{700}}) + (\mathbf{A_{512}} - \mathbf{A_{700}})] * \textbf{dilution factor} \tag{5}$$

with absorbancies measured in the sample mixed with metabisulfite