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Modeling of thermal degradation kinetics of the *C*-glucosyl xanthone mangiferin in an aqueous model solution as a function of pH and temperature and protective effect of honeybush extract matrix

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ABSTRACT

Mangiferin, a *C*-glucosyl xanthone, abundant in mango and honeybush, is increasingly targeted for its bioactive properties and thus to enhance functional properties of food. The thermal degradation kinetics of mangiferin at pH 3, 4, 5, 6 and 7 were each modeled at five temperatures ranging between 60 and 140 °C. First-order reaction models were fitted to the data using non-linear regression to determine the reaction rate constant at each pH-temperature combination. The reaction rate constant increased with increasing temperature and pH. Comparison of the reaction rate constants at 100 °C revealed an exponential relationship between the reaction rate constant and pH. The data for each pH were also modeled with the Arrhenius equation using non-linear and linear regression to determine the activation energy and pre-exponential factor. Activation energies decreased slightly with increasing pH. Finally, a multi-linear model taking into account both temperature and pH was developed for mangiferin degradation. Sterilization (121 °C for 4 min) of honeybush extracts dissolved at pH 4, 5 and 7 did not cause noticeable degradation of mangiferin, although the multi-linear model predicted 34% degradation at pH 7. The extract matrix is postulated to exert a protective effect as changes in potential precursor content could not fully explain the stability of mangiferin.

1. Introduction

Xanthones form a phenolic sub-class that is both privileged and unique. Their distribution in nature is fairly limited, with the majority of natural xanthones found in just two families of higher plants – the Guttiferae and Gentianaceae (as reviewed by Vieira & Kijjoa, 2005). Structurally, the xanthones are characterized by a basic dibenzo-γ-pyrone (9*H*-xanthen-9-one) structure, with varying degrees of hydroxylation, methoxylation, alkylation, prenylation, geranylation and/or glycosylation. The occurrence of xanthones in the genus *Cyclopia* (Family: Fabaceae) is of interest given the utilization of *Cyclopia* as honeybush herbal tea, and source of food ingredient and nutraceutical extracts (Joubert, Joubert, Bester, De Beer, & De Lange, 2011). Schulze et al. (2015) recently demonstrated that honeybush herbal tea, prepared from a number of *Cyclopia* species, represents a rich source of the *C*-glucosyl xanthones, mangiferin and isomangiferin, and could therefore contribute substantially to their dietary intake.

Mangiferin, most notably present in mango fruit and mango by-products (Berardini, Fezer, & Conrad, 2005; Dorta, González, Lobo, Sánchez-Moreno, & De Ancos, 2014), has gradually risen in importance in light of its plethora of medicinal properties. Mangiferin is known to exert a wide range of biological properties including antioxidant, anti-inflammatory and antidiabetic activities as evident from several recent reviews (Benard & Chi, 2015; Imran et al., 2017; Saha, Sadhukhan, & Sil, 2016). The α-glucosidase inhibitory activity of this xanthone (Beelders, Brand, et al., 2014) would strengthen its potential application as a natural anti-obesity and antidiabetic agent.

Mangiferin-rich extracts from natural sources therefore have tremendous potential for commercialization. Knowledge regarding the thermal stability of this xanthone is required to predict losses resulting from exposure to elevated temperatures during various unit operations in their manufacture (e.g. extraction, concentration and spray-drying) and during incorporation into food products (e.g. baking, extrusion processing, pasteurization/sterilization). Thus far, investigations of

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thermal stability of mangiferin have been very limited. In a recent study on *C. genistoides* plant material, containing high levels of the compound (Beelders, De Beer, & Joubert, 2015), we demonstrated that mangiferin is susceptible to extensive thermal degradation upon high-temperature chemical oxidation (90 °C for 16 h) of the plant material, an essential step for development of the characteristic sensory profile of this herbal tea. It was found that the degradation of mangiferin may adequately be described by first-order degradation kinetics. In a further study (Beelders, De Beer, Ferreira, Kidd, & Joubert, 2017), an aqueous model solution of mangiferin at pH 5 was employed to gain insight into its thermal stability and degradation products without the interference of other compounds. For instance, 3-β-D-glucopyranosylmaclurin, present in *C. genistoides*, can be converted to mangiferin, confounding mangiferin degradation rates. The temperature-dependence of its degradation was determined at a pH of 5, selected to mimic that of herbal tea. Another study found that thermal degradation of mangiferin occurs in very alkaline solution (0.1 M NaOH), while no degradation was observed in very acidic solution (0.1 and 1 M HCl) (Khurana, Kaur, Kaur, & Singh, 2017).

The aim of the present work was to kinetically assess the thermal stability of mangiferin in aqueous model solutions at varying pH levels (pH 3–7) as other pH values are also relevant in food processing, depending on the product. Insight into its stability in solution as affected by pH could guide process control and product development. Aqueous solutions of mangiferin were subjected to thermal treatment at five different temperatures for each pH level, and its degradation followed by ultra-high performance liquid chromatography coupled with diode-array detection (UHPLC-DAD). The degradation products were characterized by coupling of the optimized LC method to electrospray ionization mass spectrometry (ESI-MS) and tandem MS detection. The reaction rate constants for the degradation of mangiferin under the various conditions were computed using appropriate mathematical models. Hereby, the pH- and temperature- dependence of the degradation rate constants was also characterized mathematically. In order to determine whether the model generated from data in model solutions could be extrapolated to food products, two honeybush (*C. genistoides*) extracts were heated under sterilisation conditions (121 °C for 4 min) after reconstitution in buffer solutions at pH 4, 5 and 7. These pH values represent typical values comparable to an iced tea containing citric acid, a natural iced tea with no acidification and a milk-based drink, respectively.

2. Materials and methods

2.1. Chemicals

General reagent grade laboratory chemicals and the authentic reference standard for mangiferin were procured from Sigma-Aldrich (St. Louis, MO, USA). HPLC gradient-grade acetonitrile and formic acid (98–100%) were purchased from Merck Millipore (Darmstadt, Germany). HPLC-grade water was prepared using Elix Advantage 5 UV and Milli-Q Reference A+ water purification systems (Merck Millipore) in series. A stock solution (ca. 6 mM) of mangiferin was prepared in 100% dimethylsulphoxide (DMSO) and aliquots frozen at −20 °C until further use.

2.2. Thermal degradation kinetics experiments

Thermal degradation experiments were conducted as described by Beelders et al. (2017) at five temperatures for each pH level (pH 3, 4, 5, 6 and 7). The temperature range at each pH spanned 50 °C with 10 °C intervals. All ranges fell between 60 and 140 °C. The temperature ranges employed for each pH were selected to accommodate the thermal stability of mangiferin as determined by preliminary experiments.

For each temperature-pH combination, the mangiferin stock solution in DMSO was diluted with 0.1 M phosphate buffer solution, adjusted to the required pH with 0.1 M HCl or 0.1 M NaOH, to obtain a 0.1 mM working solution. Aliquots (0.8 mL) were sealed in 5 mL glass micro reaction vials (Supelco, Bellefonte, PA, USA). Of these, one served as unheated control while the remaining vials (n = 24) were placed in a pre-heated Stuart heating block equipped with digital temperature control. Each of the individual holes in the heating block contained ca 1.5 mL of glycerin to improve heat transfer.

Replicate samples (n = 3) were removed from random positions in the heating block at predetermined time points (n = 8) and cooled for 15 min in an ice bath. They were filtered (0.22 µm pore size, 4 mm diameter Millex-GV syringe filters; Merck Millipore) and an aliquot of each removed for immediate analysis by UHPLC-DAD after addition of aqueous ascorbic acid (final concentration = 9.1 mg/mL). Aliquots were also frozen at −20 °C until LC-MS analysis.

2.3. Quantification of mangiferin using UHPLC-DAD

UHPLC-DAD analysis was conducted on an Agilent 1290 UHPLC instrument equipped with an in-line degasser, binary pump, autosampler, column thermostat and diode-array detector controlled by OpenLab Chemstation software (Agilent Technologies Inc., Santa Clara, CA, USA). Samples (10 µL) were separated on an Agilent Zorbax Eclipse Plus C_{18} column (Rapid Resolution HD; 1.8 µm, 2.1 × 50 mm), thermostatted to 23 °C, using a solvent gradient of (A) 0.1% formic acid in water (v/v) and (B) acetonitrile at 0.7 mL/min: 5–22% B (0–2.2 min), 22–50% B (2.2–2.6 min), 50% B (2.6–4.1 min), 50–5% B (4.1–4.6 min), 5% B (4.6–6.6 min) (Beelders et al., 2017). A standard calibration solution comprising mangiferin (0.0363 µg/µL) and ascorbic acid (ca 9.5 µg/µL) in HPLC-grade water was filtered using 0.22 µm pore-size Millex-GV syringe filters (4 mm diameter, Millipore) prior to injection (0.5–15 µL). UV spectra were recorded between 200 and 500 nm at an acquisition rate of 20 Hz. The mangiferin content of the samples was quantified using the peak area at 320 nm. For method validation purposes, the stability of mangiferin in 0.1 M phosphate buffer solution at pH 3, 4, 5, 6 and 7 was monitored at room temperature by repeat injections over a 12 h period (n = 6; v_{inj} = 7 µL) (Table A.1, Supplementary material).

Univariate analysis of variance (ANOVA) using the General Linear Models Procedure was performed on the data sets for each pH-temperature combination separately (SAS, version 9.2; SAS Institute, Cary, NC, USA). The Shapiro-Wilk test was performed to test for normality. Fisher's least significant difference was calculated at the 5% level ($p < 0.05$) to compare means across treatment times. The average concentrations of mangiferin as a function of treatment temperature and time at the respective pH values are supplied in Tables A.2–A.6 (Supplementary material).

2.4. Identification of thermal degradation products by LC-DAD-ESI-MS and -MS/MS

One sample of mangiferin thermally treated at 100 °C at each of the respective pH values was selected for LC-DAD-ESI-MS and -MS/MS analyses. These analyses were conducted on an Acquity UPLC system equipped with a binary solvent manager, sample manager, column heating compartment and photodiode-array detector coupled to a Synapt G2 Q-TOF system equipped with an electrospray ionization source (Waters; Milford, MA). Front end separation was achieved according to the UHPLC method described in Section 2.3. Data were acquired in resolution (scanning from 150 to 1500 amu) and MS/MS scanning modes and processed using MassLynx v.4.1 software (Waters). The instrument was operated in positive and negative ionization modes and calibrated using a sodium formate solution. Leucine enkephalin was used for lockspray (ESI^+, lock mass 556.2771 amu; ESI^-, lock mass 554.2615 amu). The MS parameters were as follows: capillary voltage 2.5 kV, sampling cone voltage 15.0 V, source temperature