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Effect of dielectric microwave heating on the color and antiradical capacity of betanin

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ABSTRACT

The decomposition of betanin under dielectric heating (microwave irradiation, power: 25–200 W (3–24 kJ g^{-1})) follows first-order kinetics with a rate constant similar to that obtained during conventional conduction heating (half-life < 2 min at 100 °C). Color coordinate analysis indicates that betanin is bleached upon thermal treatment, whereas beetroot juice and spray-dried beetroot powder tend to form colored decomposition products. The antiradical capacity of betanin decreases upon heating, but is still much higher than that of standard antioxidants such as ascorbic acid and trolox. Betalamic acid, a high capacity antiradical, was detected by mass spectrometry and second-derivative absorption spectroscopy in betanin samples submitted to thermal treatment.

1. Introduction

Betalains are colored natural products of increasing importance as nutraceutical agents due to their antioxidant, antiviral and antimicrobial activities (Delgado-Vargas and Paredes-López, 2002; Ravichandran et al., 2012; Wootton-Beard and Ryan, 2011). Sources of betalains used for food-coloring purposes contain, among other substances, a mixture of betanin (**Bn**, 2*S*/15*S*) and its epimer, isobetanin (**iBn**, 2*S*/15*R*). Betanin (CI Natural Red 33, E-code E162, h° = 336, betanidin 5-*O*-β-glucoside, Scheme 1) is obtained almost entirely from red beet crops and is the only betalain approved for use in food (Delgado-Vargas et al., 2000; Delgado-Vargas and Paredes-López, 2002; Gonçalves et al., 2012). Furthermore, betanin has been used in technological applications such as solar cells (Sandquist and McHale, 2011; Zhang et al., 2008), and the development of fluorescent probes for the live-imaging of *Plasmodium* (Gonçalves et al., 2013).

The analysis of color changes in betalainic foodstuff is often used to study the degradation pathways of betalains (Herbach et al., 2004a,b, 2006a; von Elbe and Maing, 1973). Combined with high water activity (Bartoloni et al., in press), temperature is the most critical factor related to betalain stability, and the products responsible for particular color alterations have been characterized by a combination of spectrophotometry, mass spectrometry (MS) and high-performance liquid chromatography (HPLC) measurements (Herbach et al., 2006a). Upon heating, the color of red beet juice samples changes to orange–yellow, mostly due to oxidation (i.e., dehydrogenation in the presence of oxygen), hydrolysis and decarboxylation of betanin. Other processes such as epimerization and deglucosylation are equally important (Scheme 1) (Delgado-Vargas et al., 2000; Herbach et al., 2004a,b, 2006b). The formation of decarboxylated and dehydrogenated degradation products during heat treatment of betanin has already been reported to occur in purple pitaya and red beet juices (Herbach et al., 2004a,b; Wybraniec, 2005).

The chemical modification of food is often related to color changes, which might compromise commercial appeal, health benefits and safety. The dielectric heating of polarizable molecules with microwave irradiation has been used in food chemistry mostly for drying and extraction purposes (Gonzalez-Nunez and Canizares-Macias, 2011; Li et al., 2012). Microwave irradiation is also a milestone technique in food cooking and heating. However, microwaves favor polymerization, epimerization and oxidation of polyphenolic antioxidants (Carta and Loddo, 2002). Therefore, dielectric heating could potentially change the chemical properties of betalains faster and in a different manner compared with conventional conduction heating.

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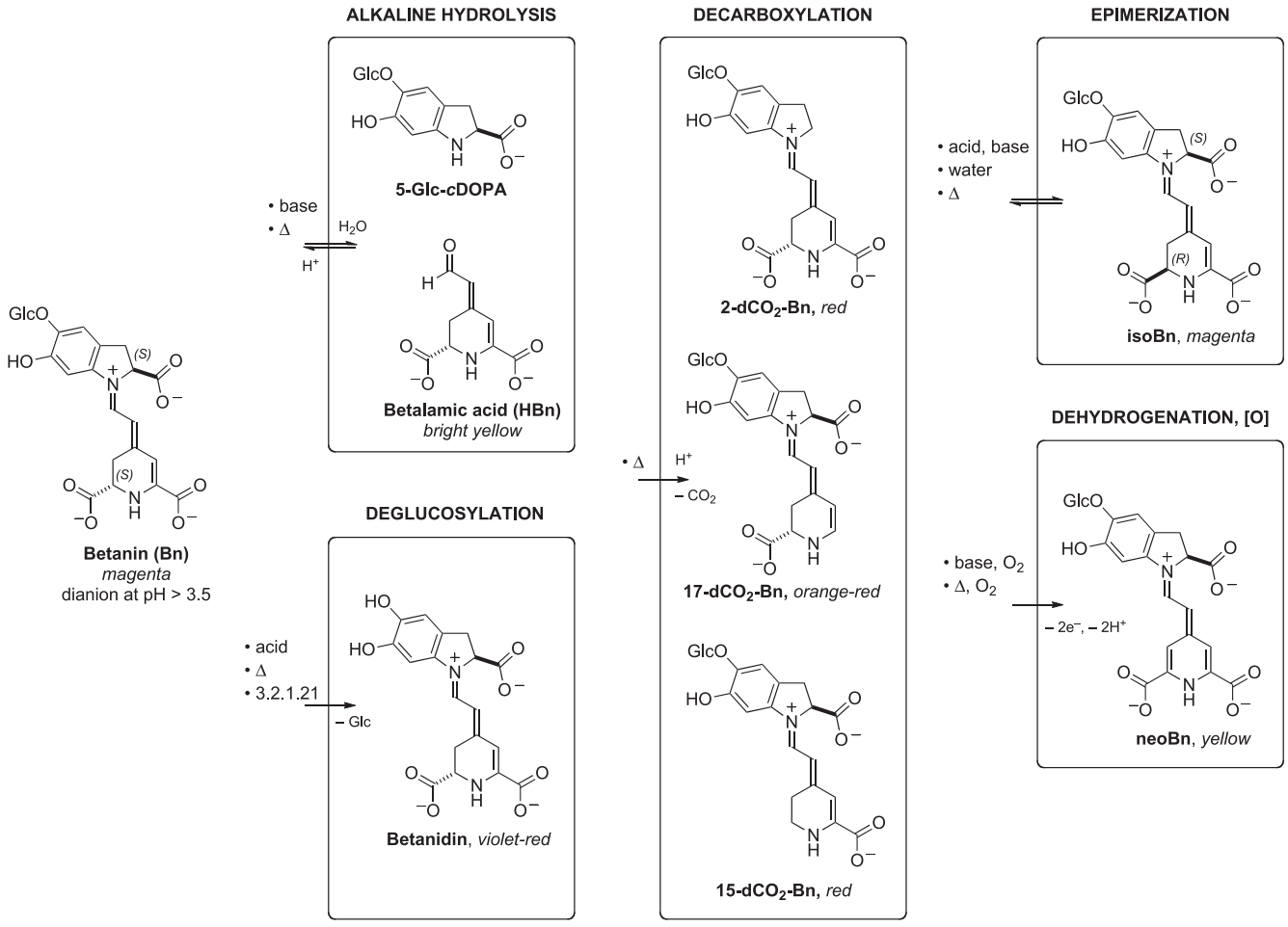

Scheme 1. Betanin degradation products and the corresponding degradation processes. dCO2 = decarboxy, neoBn = neobetanin, iBn = (2*S*/15*R*)-betanin.

In this study, we examine the effect of dielectric heating on the color and antiradical capacity of purified betanin, commercial food-grade spray-dried beetroot powder and beetroot juice. The effect of microwave irradiation power is examined using a specific experimental approach designed to compare dielectric and conduction heating.

2. Materials and methods

2.1. Chemicals

Trifluoroacetic acid (TFA), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), sodium phosphates (Na_3PO_4, Na_2HPO_4 and NaH_2PO_4), sodium hydroxide (NaOH), potassium persulfate ($K_2S_2O_8$) and silica gel 90 C18-RP (230–400 mesh) were obtained from Sigma–Aldrich. HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were obtained from Merck. All solutions were prepared using deionized water (18.2 MΩ cm, Milli-Q, Millipore).

2.2. Sources of betanin

Beetroots (*Beta vulgaris* subsp. *vulgaris* var. *vulgaris*, 0.5 kg) were peeled, sliced and homogenized using a centrifugal juice extractor (Phillips–Walita, RI1858) operated at the maximum speed. The juice was centrifuged (1370*g*, 30 min, 25 °C) and filtered (Whatman qualitative filter paper, grade 4), and the supernatant was stored at −20 °C and used within 5 days. The betanin/isobetanin mixture was purified from beetroot juice by reversed-phase column chromatography (silica gel 90 C18-RP, 20 g, conditioned and eluted with deionized water at a flow rate of 0.3 mL min^{-1}). The concentration of betanin was determined by assuming a molar absorption coefficient (ε) of 6.5×10^4 L mol^{-1} cm^{-1} at 536 nm (Schwartz and von Elbe, 1980). A suspension of food-grade spray-dried beetroot in water (200 mg mL^{-1}) was filtered with a polytetrafluoroethylene (PTFE) filter membrane (25 mm, pore size 0.45 μm), and the resultant solution was stored at −20 °C and used within 5 days. The concentration of betanin/isobetanin in the beetroot juice and in the spray-dried beetroot was determined by quantitative HPLC as described elsewhere (Gonçalves et al., 2012).

2.3. Microwave irradiation

A phosphate buffer solution (PiB, 19 mL, pH = 7.4, 0.1 mol L^{-1}) was placed in a 50 mL one-necked round-bottom flask fitted with a Claisen head, which contained a PTFE syringe tube and a thermometer and was connected to a water-cooled condenser (Fig. S1). The buffer was heated under magnetic stirring in the internal cavity of a monomode microwave reactor (CEM Discovery) until the temperature was equilibrated (typically 30–45 min, 100 ± 3 °C, Fig. S2). The solution temperature was monitored simultaneously using both a non-contact infrared (IR) thermometer located below the reaction flask and a thermometer fitted to the Claisen head. Next, 1 mL of a PiB solution containing betanin (absorbance at 536 nm = 1.0 at 25 °C) was added to the refluxing buffer through a syringe coupled to the PTFE tube. Aliquots (100 μL) were sampled after 0.3, 2.5, 5.0, 10, 20 and 40 min of irra-