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.

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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, 2S/15S) and its epimer, isobetanin (iBn, 2S/15R). Betanin (CI Natural Red 33, E-code E162, λ = 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 and Paredes-López, 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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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 (Na₃PO₄, Na₂HPO₄ and NaH₂PO₄), sodium hydroxide (NaOH), potassium persulfate (K₂S₂O₈) 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 (1370g, 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 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 × 10⁴ 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-
diation at a constant power of 25, 50, 100 or 200 W corresponding to total energies of 3, 6, 12 or 24 kJ g\(^{-1}\), respectively. The change in volume (total aliquot volume = 0.6 mL or 3% (v/v)) did not alter the temperature inside the reaction flask, and aliquots could be withdrawn using the same PTFE syringe tube without compromising the thermal equilibrium. Control experiments were carried out using the same procedure and apparatus except that a mantle (maximum output power of 130 W) was used as the heat source.

2.4. Absorption spectroscopy and color analysis

Absorption spectra were recorded in the visible region of the electromagnetic spectrum (380–780 nm) at 25 ± 1 °C on a Varian Cary 50 Bio spectrophotometer equipped with a Peltier-thermostatted cell holder. Color analyses were performed using the CIE \(L^a\) b\(^*\) D-65/2\(^*\) color space in the Color software (v.3.1, Startek Technology). \(L^a\) indicates the lightness, and its value ranges from 0 (an ideal black object) to 100 (an ideal white object). In the CIE \(L^a\) b\(^*\) \(b^*\) system, \(a^*\) and \(b^*\) are the chromaticity coordinates. A positive \(a^*\) value indicates the red direction, and a negative \(a^*\) value is the green direction. A positive \(b^*\) value is the yellow direction, and a negative \(b^*\) value is the blue direction. Two derived color parameters, the hue angle \((h = \arctan(b^*/a^*))\) and the Chroma value \((C = (a^2 + b^2)^{1/2})\), were calculated.

2.5. Antiradical capacity

The antiradical capacity was determined by the ABTS\(^+\) radical scavenging spectrophotometric assay (Re et al., 1999). Briefly, a stock solution of ABTS\(^+\) was prepared by the oxidation of ABTS (7 mmol L\(^{-1}\)) with \(K_2S_2O_8\) (140 mmol L\(^{-1}\)) in water at room temperature for 16 h, during which the solution was kept in the dark. The stock solution of ABTS\(^+\) was diluted in a phosphate buffer, \(pH = 7.4\) (0.1 mol L\(^{-1}\)), to an absorbance of 0.7 at 734 nm. An aliquot of the analyte solution (20–60 \(\mu\)L) or a Trolox standard was added to the ABTS\(^+\) solution in a quartz cuvette (10 mm optical path, final volume = 2 mL, [antiradical] = 1 mmol L\(^{-1}\)), and the variation in the absorbance at 734 nm was measured after a reaction period of 6 min (AA). The antiradical capacity of the sample was expressed as the Trolox equivalent antioxidant capacity (TEAC), which was determined from the \(\Delta A_{\text{sample}}/\Delta A_{\text{trolox}}\) ratio.

2.6. Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis

Analytical RP-HPLC analyses were performed using a Waters 2695 Alliance system instrument with a UV–Vis detector (dual-wavelength, Waters 2489) and a Supelcosil LC-18 column (L x ID: 150 x 4.6 mm, 5 μm; Supelco). Analysis conditions: the mobile phase was composed of solvent \(A\): 0.1% (v/v) TFA in water, and solvent \(B\): 60% (v/v) MeCN/water with 0.1% (v/v) TFA. Elution was as follows: linear gradient from 5% to 95% B in 20 min at 25 °C; flow rate was set to 1 mL min\(^{-1}\). The spectrophotometric detection was set at 254/536 nm for \(Bn\). The injection volume was set to 10 μL.

2.7. HPLC-DAD-ESI(+)–MS/MS analysis

Analytical HPLC-MS analyses were performed using a Bruker Daltonics Esquire HCT ion trap mass spectrometer equipped with an electrospray source (positive mode, ESI) that was coupled to a Shimadzu Prominance liquid chromatograph. The chromatograph was equipped with a Luna C18 column (L x ID: 150 x 2.1 mm, 3 μm; Phenomenex) maintained at 30 °C and a Shimadzu SPD-M20A diode array detector (DAD). Analysis conditions are the same used for RP-HPLC experiments. Nitrogen was used as the nebulizing (45 psi) and drying gas (6 L min\(^{-1}\), 300 °C), and helium was used as the buffer gas (4 x 10\(^{-6}\) mbar). The capillary high voltage was set to 3500 V. To avoid space-charge effects, the smart ion charge control (ICC) was set to an arbitrary value of 50,000.

2.8. Kinetic studies

Rate constants were determined using a monoexponential decay (first-order rate) model (Eq. (1)). Non-linear fitting of the data required the inclusion of a term \(y_0\) to account for the residual absorbance at 536 nm after thermal treatment.

\[ y = y_0 + Ae^{-kt} \]  

(1)

2.9. Statistical analysis

All values are expressed as the mean ± standard deviation (sd) of three completely independent replicates. Statistical data analyses were performed using one-way analysis of variance (ANOVA). The level of statistical significance was set to \(p < 0.05\).

3. Results and discussion

3.1. Decomposition kinetics and color profile

The effects of conventional and microwave dielectric heating on a chemical process are easier to compare when superheating effects and differences in thermal stabilization are avoided (Kappe, 2004). For this purpose, we employed an experimental apparatus suitable for heating \(Bn\) solutions under identical experimental conditions using either a microwave source (dielectric heating) or a heating mantle (conventional heating). It consisted of a round-bottomed flask with a Claisen head. One opening of the Claisen head was fitted with a rubber septum penetrated by a thermometer and PTFE syringe tubing, and the other opening was attached to a water-cooled condenser (Fig. S1).

The effects of the heating method and the microwave irradiation power on the thermal decomposition of \(Bn\) in refluxing PiB were investigated. Both microwave irradiation (25–200 W) and conventional heating of the \(Bn\) solutions resulted in a monoexponential decrease of the absorption at 536 nm, which followed first-order kinetics (Fig. S3). This behavior is in agreement with that reported for the thermal decomposition of \(Bn\) and other betacyanins in red beet and purple pitaya juices during conventional heating (Herbach et al., 2004b; von Elbe et al., 1974).

The rate constants and half-lives of \(Bn\) at 100 °C are given in Table 1. Under the experimental conditions used in this study, the irradiation power and method of heating had no effect on the decomposition rate constants. Furthermore, the experimental half-life of \(Bn\) in a refluxing PiB solution was approximately 1.8 min, which agrees with that calculated from the activation energy for the decomposition of \(Bn\) in Mcllvaine’s buffer \((T_g = 1.9\) min, \(E_a = 144.8\) kJ mol\(^{-1}\), \(pH = 5.0\)) (Saguy et al., 1978). The reaction flask used in this study had a diameter of 4.5 cm, and the penetration depths of microwaves in water are 1.4 and 5.7 cm at 25 °C and 95 °C, respectively (Bodgal, 2005). Therefore, the lack of microwave effects might be related to the irradiation of a system that was already in thermal equilibrium.

Considering these results, we investigated the decomposition kinetics and the changes in the color profiles of \(Bn\), commercial food-grade spray-dried beetroot powder and fresh beetroot juice solutions during thermal treatment by dielectric heating at a fixed irradiation power of 200 W (24 kJ g\(^{-1}\)). These samples represent the main betalainic sources used commercially for food coloring purposes in Europe and North America (Stintzing and Carle, 2008b). Aliquots were immediately diluted in PiB at room temper-
nature and analyzed by direct (zero order) and second-derivative Vis spectroscopy (Fig. 1). Samples containing mixtures of betalains show complex absorption profiles; therefore, the identification of single components requires the use of complementary analytical methods, such as HPLC-DAD-MS/MS (Herbach et al., 2006b). Pure Bn shows a split Gaussian band with a maximum at 536 nm. Upon heating, this band decreases with time, while a second band appears at 430 nm, which most likely indicates the formation of betalamic acid (HBt) (Fig. 1A) (Herbach et al., 2005; Saguy et al., 1978). Beet-root powder has a much more complex absorption profile characterized by a broad band with shoulders at approximately 530 and 480 nm (betacyanins and betaxanthins, respectively) and high absorption in the UV region (Fig. 1B). Second-derivative analysis of the absorption data eliminates the baseline curvature and improves the band resolution, provided the spectra can be fitted to a quadratic equation with respect to wavelength. Therefore, we analyzed the second-derivative spectra of the beetroot powder (Fig. 1B). The spectra indicate the presence of superimposed bands with three main signals at 540, 480 and 450 nm (Fig. 1B). The band at 480 nm disappeared more quickly than that at 540 nm upon thermal treatment. This result is in agreement with the lower thermal stability of betaxanthins (e.g., vulgaxanthin I) compared to betacyanins (Herbach et al., 2004a; Sapers and Hornstein, 1979; Stintzing and Carle, 2008b). No evidence of HBt formation was observed upon thermal treatment of beet powder. In contrast, for beetroot juice (Fig. 1C), the shoulder at 480 nm was much less intense, and a band at 430 nm was formed upon heating, most likely indicating the presence of HBt. The drying of beetroot juice under microwave irradiation at 1 atm (Fig. 1C, dotted line) resulted in an absorption profile similar to that of beet powder after irradiation (Fig. 1B), with the exception of a prominent band at approximately 450 nm.

The color changes caused by the thermal treatment of betanin, beet powder and beetroot juice solutions were determined from absorption data using the CIE L’/C3a/C3b color space (Table S2). Fig. 2 depicts the changes in redness (a’), and yellowness (b’); the derived parameters, Chroma (C’) and hue angle (h’); and the lightness value (L’). Color monitoring must be performed using the same dilution of the samples to track changes in L’, h’ and C’ (Stintzing and Carle, 2008a). Therefore, solutions of the three samples were prepared in PiB (pH = 7.4), and the optical densities at 536 nm were adjusted to 1.0; however, the amount of betanin was different in each sample due to the complex composition of beetroot powder and juice (Gonçalves et al., 2012).

The degradation of Bn was accompanied by an increase in L’ and a decrease in h’ (Fig. 2). These results are in general agreement with previously reported data for the thermal decomposition caused by conventional heating (Herbach et al., 2004a,b, 2006a,b; von Elbe and Maing, 1973). Exponential fitting of the L’ data results

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**Table 1**

First-order rate constants and half-lives (τ<sub>1/2</sub>) of Bn solutions subjected to thermal treatment.

<table>
<thead>
<tr>
<th>Bn, Control</th>
<th>0.004</th>
<th>0.003</th>
<th>0.17</th>
<th>0.01</th>
<th>0.43</th>
<th>0.04</th>
<th>1.6</th>
<th>0.9997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bn, 25 W</td>
<td>0.008</td>
<td>0.005</td>
<td>0.18</td>
<td>0.01</td>
<td>0.40</td>
<td>0.08</td>
<td>1.8</td>
<td>0.9850</td>
</tr>
<tr>
<td>Bn, 50 W</td>
<td>0.006</td>
<td>0.004</td>
<td>0.15</td>
<td>0.01</td>
<td>0.36</td>
<td>0.04</td>
<td>1.9</td>
<td>0.9999</td>
</tr>
<tr>
<td>Bn, 100 W</td>
<td>0.006</td>
<td>0.004</td>
<td>0.15</td>
<td>0.01</td>
<td>0.37</td>
<td>0.03</td>
<td>1.9</td>
<td>0.9970</td>
</tr>
<tr>
<td>Bn, 200 W</td>
<td>0.005</td>
<td>0.004</td>
<td>0.20</td>
<td>0.01</td>
<td>0.41</td>
<td>0.04</td>
<td>1.7</td>
<td>0.9907</td>
</tr>
</tbody>
</table>

* Fitted using Eq. (1).
* sd = standard deviation (N = 3). Bn = betanin.

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Fig. 1. Effect of microwave heating on the absorption (bottom) and second derivative spectra (top) of phosphate buffer solutions (pH = 7.4) of betanin (A), spray dried beetroot powder (B) and fresh beetroot juice (C) with absorbance = 1.0 at 536 nm. Dashed lines indicate the absorption at room temperature. Arrows indicate the decrease in absorption at 536 nm with time. The decreasing dotted line in (C) corresponds to the absorption profile of microwave-dried (1 atm) beetroot juice.
in a rate constant comparable to that determined by monitoring the absorbance at 536 nm \( (k_{536} \approx k_{L} \approx 0.4 \text{ min}^{-1}) \). Likewise, the rate constants determined from the absorbance measurements of beetroot powder and juice solutions at 536 nm (0.33 ± 0.04 and 0.32 ± 0.05 min\(^{-1}\), respectively, Table S1 and Fig. S3) are close to those determined from the increase in lightness \( (k_{L} \approx 0.28 \text{ min}^{-1}) \). The \( L^* \) value becomes constant at approximately 90 for heated samples of beetroot powder and juice, indicating that they are darker than \( Bn \), most likely due to the formation of browning substances (Saguy et al., 1978). This hypothesis is corroborated by the higher values of the limiting absorbance, \( y_0 \) (Fig. S3), for beetroot powder and juice (0.12 and 0.08, respectively, Table S1) compared to that for \( Bn \) (<0.05, Table 1). The increasing \( L^* \) values reflect the decrease in the concentration of betacyanins (Herbach et al., 2006b). The \( L^* \) values of beetroot powder and beetroot juice are nearly identical, but they are lower than that of betanin, reflecting the higher stability of betalains in complex matrices.

The beetroot juice and \( Bn \) solutions were more violet (shades of magenta) than the beetroot powder samples, whose color was less red and shifted more toward yellow (Fig. 2, parameters \( a^* \) and \( b^* \)). Upon heating, the beetroot juice and powder solutions became bright yellow, whereas the \( Bn \) solutions were bleached \( (a^* \approx b^* \approx 0) \). These results indicate that the beetroot juice solutions in \( Pib \) undergo a more expressive color change upon thermal treatment. The hue angles of \( Bn \) and beetroot juice decreased during the first 5 min of heating \( (0 < h^* < 60, \text{red to yellow}) \) and, after a slight increase, became constant at approximately 100°, which corresponds to a yellow–green color (Fig. 2). For beet powder, \( h^* \) showed a saturation profile, also reaching a plateau at approximately 100°. It must be noted that the high values of \( L^* \) influenced the color saturation, and therefore, the color of the solutions was close to yellow.
Betacyanin degradation is accompanied by decreasing C values (Herbach et al., 2006b). However, the thermal treatment of red beet juice, which contains a mixture of betacyanins and betaxanthins, was reported to increase C due to the incremental formation of differently colored compounds (Herbach et al., 2006b; Stintzing and Carle, 2008a). Accordingly, pure Bn showed an abrupt decrease in C from 73–3 and an increase in yellowness (b' and L') in the first 5 min of thermal treatment. These changes resulted in a low color purity and high luminosity (i.e., loss of color) due to pigment decomposition, independent of the heating method. These results contrast with those reported for the irradiation of beet root tissue immersed in water with a microwave power of up to 450 W (Latorre et al., 2012). However, for beetroot powder and juice, the C values decreased and became constant at values of approximately 25 and 11, respectively, indicating the formation of other colored substances. This result can be explained by the formation of brown compounds through Maillard reactions, as described previously for betalains and anthocyanins subjected to conventional heating during processing (Wojdyło et al., 2009). The decomposition of polyphenol oxidases and peroxidases of B. vulgaris L. var. conditiva during thermal treatment using microwave irradiation (Latorre et al., 2012) might indicate that enzymatic browning (Gandia-Herrero et al., 2005) plays a minor role in the higher C values of both beetroot juice and powder compared to that of Bn.

### 3.2. Antiradical capacity

The effect of the thermal treatment on the antiradical capacity of Bn is given in Fig. 3. We used the ABTS⁺ colorimetric assay to determine the antiradical capacity. The results are reported in Trolox equivalents because of the large amount of data available for other natural antioxidants (Rice-Evans et al., 1996). Beetroot juice and beet powder were not tested due to their complex compositions, which make the results difficult to interpret. For example, commercial spray-dried beetroot powder contains other antioxidants apart from betalains (such as citric acid or ascorbic acid) (Herbach et al., 2006a; Stintzing and Carle, 2008b) and the betalain distribution in beetroot juice depends on the cultivar (Czapski et al., 2009).

The TEAC of pure Bn in PIB was determined to be 4.9 ± 0.7, which is in agreement with the value reported in the literature (TEAC = 4.5) (Gandia-Herrero et al., 2010). The antiradical capacity of Bn decreased upon both dielectric and conventional heating until a plateau was reached at a TEAC value of approximately 3.3, which corresponds to an average decrease in the TEAC value of 30%, after a 20 min period. However, although the TEAC values of the decomposition products stabilized after 10 min of reflux, their antiradical capacity was still very high after 40 min of heating compared to those of other antioxidants, such as ascorbic acid (TEAC = 0.99 ± 0.03 (Rice-Evans et al., 1996), 1.05 ± 0.03 (Re et al., 1999)), rutin (TEAC = 2.4) (Rice-Evans et al., 1996) and epicatechin (TEAC = 2.5) (Rice-Evans et al., 1996). Furthermore, although it seems from Fig. 3 that the TEAC values determined during conventional and dielectric heating are different, this apparent difference is not statistically significant at the p < 0.05 level.

The high TEAC values of Bn samples subjected to thermal treatment might be related to the formation of HBt, which was inferred from the absorption data. To verify this hypothesis, we monitored the change in the absorption band at 434 nm using second-derivative spectroscopic data (Fig. 4A). Furthermore, HPLC/DAD-ESI(+)—MS/MS analyses were performed before and after microwave irradiation of Bn in an attempt to identify the decomposition products (Fig. 4B).

The HPLC/MS analyses indicate that HBt is formed during conventional and dielectric heating of Bn. The absorption data show that the HBt concentration peaked after 10 min of irradiation and then began to decay. This result can be explained by the partial regeneration of Bn (Herbach et al., 2005), which also explains the near-constant TEAC value after this period. The TEAC of betalamic acid at pH = 7.0 was reported to be 2.7, despite the fact that it is difficult to obtain and characterize this unstable aldehyde (Gandia-Herrero et al., 2012). The presence of other substances resulting from the irradiation of Bn, such as cyclo-DOPA (cyclo L-3,4-dihydroxyphenylalanine) derivatives, might be responsible for the slightly higher TEAC value reported here.

### 4. Conclusions

Under the conditions used in this study, conduction and dielectric heating have the same effect on the decomposition rate of Bn, regardless of the microwave irradiation power used. In solution, pure betanin is bleached by thermal treatment, whereas for beetroot juice and spray-dried beet powder, heating favors the formation of colored products (i.e., browning), which partially maintain the solution color. The antiradical capacity of Bn is decreased by thermal treatment but is higher than that of Trolox. Mass spectrometry analysis indicates that betalamic acid is formed during conventional or dielectric heating of Bn.
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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfoodeng.2013.03.022.

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